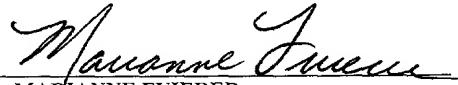


|  |  |   |   |
|--|--|---|---|
| FORM PTO-1390  |  | U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE | ATTORNEY'S DOCKET NUMBER  |
| TRANSMITTAL LETTER TO THE UNITED STATES<br>DESIGNATED/ELECTED OFFICE (DO/EO/US)<br>CONCERNING A FILING UNDER 35 U.S.C. 371   |  |   | 4121-126  |
|  |  |   | U.S. APPLICATION NO. (If known, see 37 CFR 1.5)<br><b>09/889182</b> |
| INTERNATIONAL APPLICATION NO.<br>PCT/DE00/00079  | INTERNATIONAL FILING DATE<br>11 January 2000 | PRIORITY DATE CLAIMED<br>11 January 1999                |   |
| TITLE OF INVENTION<br><b>SELECTION OF MONOCLONAL ANTIBODIES</b>  |  |   |   |
| APPLICANT(S) FOR DO/EO/US<br><b>Frank Breitling, Annemarie Poustka and Gerard Moldenhauer</b>  |  |   |   |
| Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:  |  |   |   |
| <ol style="list-style-type: none"> <li>1. <input checked="" type="checkbox"/> This is a <b>FIRST</b> submission of items concerning a filing under 35 U.S.C. 371.</li> <li>2. <input type="checkbox"/> This is a <b>SECOND</b> or <b>SUBSEQUENT</b> submission of items concerning a filing under 35 U.S.C. 371.</li> <li>3. <input type="checkbox"/> This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).</li> <li>4. <input checked="" type="checkbox"/> A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.</li> <li>5. <input checked="" type="checkbox"/> A copy of the International Application as filed (35 U.S.C. 371(c)(2)) <ol style="list-style-type: none"> <li>a. <input checked="" type="checkbox"/> is transmitted herewith (required only if not transmitted by the International Bureau).</li> <li>b. <input checked="" type="checkbox"/> has been transmitted by the International Bureau.</li> <li>c. <input type="checkbox"/> is not required, as the application was filed in the United States Receiving Office (RO/US).</li> </ol> </li> <li>6. <input checked="" type="checkbox"/> A translation of the International Application into English (35 U.S.C. 371(c)(2)).</li> <li>7. <input type="checkbox"/> Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3)) <ol style="list-style-type: none"> <li>a. <input type="checkbox"/> are transmitted herewith (required only if not transmitted by the International Bureau).</li> <li>b. <input type="checkbox"/> have been transmitted by the International Bureau.</li> <li>c. <input type="checkbox"/> have not been made; however, the time limit for making such amendments has NOT expired.</li> <li>d. <input checked="" type="checkbox"/> have not been made and will not be made.</li> </ol> </li> <li>8. <input type="checkbox"/> A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).</li> <li>9. <input checked="" type="checkbox"/> An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)). <b>*(Unsigned)</b></li> <li>10. <input type="checkbox"/> A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).</li> </ol> |  |   |   |
| <b>Items 11. to 16. below concern other document(s) or information included:</b>   |  |   |   |
| 11. <input type="checkbox"/> An Information Disclosure Statement under 37 CFR 1.97 and 1.98  |  |   |   |
| 12. <input type="checkbox"/> An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.   |  |   |   |
| 13. <input checked="" type="checkbox"/> A FIRST preliminary amendment.<br><input type="checkbox"/> A SECOND or SUBSEQUENT preliminary amendment.   |  |   |   |
| 14. <input type="checkbox"/> A substitute specification.   |  |   |   |
| 15. <input checked="" type="checkbox"/> A small entity statement.  |  |   |   |
| 16. <input type="checkbox"/> Other items or information: EPO Search Report in German   |  |   |   |

NOTE: This application is being filed with an unsigned Oath or Declaration under the provisions of 37 CFR § 1.53 in order that applicants may secure a filing date of July 10, 2001. Upon receipt of a "Notice to File Missing Parts - Filing Date Granted," a executed Declaration and Power of Attorney will be forwarded. The undersigned agent affirmatively states that she has been duly authorized and appointed to file this application on behalf of the applicants and applicants' assignees, and that the Declaration and Power of Attorney to be filed hereafter will confirm the undersigned agent's authorization and appointment. Applicants are considered a small entity and assignee Deutsches Krebsforschungszentrum is also considered a small entity within the meaning of 37 CFR § 1.9.

|   |              |              |            |   |  |              |  |
|---|--------------|--------------|------------|---|--|--------------|--|
| 17. <input checked="" type="checkbox"/> The following fees are submitted:   |              |              |            | CALCULATIONS  |  | PTO USE ONLY |  |
| <b>Basic National Fee</b> (37 CFR 1.492(a)(1)-(5)):<br>Search Report has been prepared by the EPO or JPO .....\$860.00  |              |              |            | JC18 Rec'd PCT/PTO 10 JUL 2001  |  |              |  |
| International preliminary examination fee paid to USPTO (37 CFR 1.482) .....\$0.00<br>No International preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2)) .....\$0.00<br>Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO .....\$1000.00<br>International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4).. .....\$0.00 |              |              |            |   |  |              |  |
| ENTER APPROPRIATE BASIC FEE AMOUNT =  |              |              |            | \$ 860.00   |  |              |  |
| Surcharge of \$130.00 for furnishing the oath or declaration later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(e)).   |              |              |            | \$  |  |              |  |
| Claims  | Number Filed | Number Extra | Rate       |   |  |              |  |
| Total Claims  | 20-20 =      | 0            | X \$18.00  | \$  |  |              |  |
| Independent Claims  | 2-3 =        | 0            | X \$80.00  | \$  |  |              |  |
| Multiple dependent claim(s) (if applicable)   |              |              | + \$270.00 | \$  |  |              |  |
| TOTAL OF ABOVE CALCULATIONS =   |              |              |            | 860.00  |  |              |  |
| Reduction by 1/2 for filing by small entity, if applicable. Verified Small Entity statement must also be filed. (Note 37 CFR 1.9, 1.27, 1.28).  |              |              |            | \$ 430.00   |  |              |  |
| SUBTOTAL =  |              |              |            | \$ 430.00   |  |              |  |
| Processing fee of \$130.00 for furnishing the English translation later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 Months from the earliest claimed priority date (37 CFR 1.492(f)).  |              |              |            | \$  |  |              |  |
| TOTAL NATIONAL FEE =  |              |              |            | \$ 430.00   |  |              |  |
| Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). \$40.00 per property  |              |              |            | \$  |  |              |  |
| TOTAL FEE ENCLOSED =  |              |              |            | \$ 430.00   |  |              |  |
|   |              |              |            | Amount to be:   |  | \$           |  |
|   |              |              |            | refunded  |  |              |  |
|   |              |              |            | Charged   |  | \$           |  |
| a. <input checked="" type="checkbox"/> A check in the amount of \$430.00 to cover the above fees is enclosed.<br>b. <input type="checkbox"/> Please charge my Deposit Account No. _____ in the amount of \$ _____ to cover the above fees. A duplicate copy of this sheet is enclosed.<br>c. <input checked="" type="checkbox"/> The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 08-3284. A duplicate copy of this sheet is enclosed.                   |              |              |            |   |  |              |  |
| NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not yet been met, a petition to revive (37 CFR 1.127(a) or (b)) must be filed and granted to restore the application to pending status.   |              |              |            |   |  |              |  |
| SEND ALL CORRESPONDENCE TO:   |              |              |            | <br>MARIANNE FUIERER<br>Registration No. 39,983 |  |              |  |
| Steven J. Hultquist<br>Intellectual Property/Technology Law<br>P. O. Box 14329<br>Research Triangle Park, NC 27709  |              |              |            |   |  |              |  |

09/889182

09/889182

| 17. <input checked="" type="checkbox"/> The following fees are submitted:   |              |              |            | CALCULATIONS                          | PTO USE ONLY |
|---|--------------|--------------|------------|---------------------------------------|--------------|
| <b>Basic National Fee (37 CFR 1.492(a)(1)-(5)):</b><br>Search Report has been prepared by the EPO or JPO .....\$860.00<br><br>International preliminary examination fee paid to USPTO (37 CFR 1.482) .....\$0.00<br>No International preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2)) .....\$0.00<br><br>Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO .....\$1000.00<br><br>International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4) .....\$0.00 |              |              |            | <b>JC18 Rec'd PCT/PTO 10 JUL 2001</b> |              |
| <b>ENTER APPROPRIATE BASIC FEE AMOUNT =</b>   |              |              |            | \$ 860.00                             |              |
| Surcharge of <b>\$130.00</b> for furnishing the oath or declaration later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(e)).  |              |              |            | \$                                    |              |
| Claims  | Number Filed | Number Extra | Rate       |                                       |              |
| Total Claims  | 20-20 =      | 0            | X \$18.00  | \$                                    |              |
| Independent Claims  | 2-3 =        | 0            | X \$80.00  | \$                                    |              |
| Multiple dependent claim(s) (if applicable)   |              |              | + \$270.00 | \$                                    |              |
| <b>TOTAL OF ABOVE CALCULATIONS =</b>  |              |              |            | 860.00                                |              |
| Reduction by 1/2 for filing by small entity, if applicable. Verified Small Entity statement must also be filed. (Note 37 CFR 1.9, 1.27, 1.28).  |              |              |            | \$ 430.00                             |              |
| <b>SUBTOTAL =</b>   |              |              |            | \$ 430.00                             |              |
| Processing fee of <b>\$130.00</b> for furnishing the English translation later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 Months from the earliest claimed priority date (37 CFR 1.492(f)).   |              |              |            | \$                                    |              |
| <b>TOTAL NATIONAL FEE =</b>   |              |              |            | \$ 430.00                             |              |
| Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). <b>\$40.00</b> per property   |              |              |            | \$                                    |              |
| <b>TOTAL FEE ENCLOSED =</b>   |              |              |            | \$ 430.00                             |              |
|   |              |              |            | Amount to be:                         | \$           |
|   |              |              |            | refunded                              |              |
|   |              |              |            | Charged                               | \$           |

- a. ☒ A check in the amount of \$430.00 to cover the above fees is enclosed.
- b. ☐ Please charge my Deposit Account No. \_\_\_\_\_ in the amount of \$ \_\_\_\_\_ to cover the above fees. A duplicate copy of this sheet is enclosed.
- c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 08-3284. A duplicate copy of this sheet is enclosed.

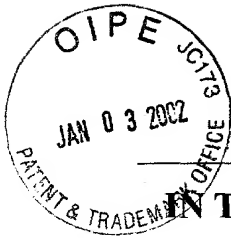
**NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not yet been met, a petition to revive (37 CFR 1.127(a) or (b)) must be filed and granted to restore the application to pending status.**

SEND ALL CORRESPONDENCE TO:

Steven J. Hultquist  
Intellectual Property/Technology Law  
P. O. Box 14329  
Research Triangle Park, NC 27709

*Marianne Fuieler*  
MARIANNE FUIERER  
Registration No. 39,983

20070913 011002



JC14 Rec'd PCT/PTO 03 JAN 2002

PCT

4121-126  
PATENT APPLICATION

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

**In re Application of:** BREITLING, et al.  
**Application No.:** 09/889,182  
**International Application No.:** PCT/DE00/00079  
**Priority Date Claimed:** 11 January 2000 and 11 January 1999 (German Appl. No. 199 00 635.0)  
**Title:** SELECTION OF MONOCLONAL ANTIBODIES



23448

PATENT & TRADEMARK OFFICE

**FIRST CLASS MAIL CERTIFICATE**

I hereby certify that I am mailing the attached documents to the Commissioner for Patents on the date specified, in an envelope addressed to the Commissioner for Patents, Washington, DC 20231, and First Class Mailed under the provisions of 37 CFR 1.8.

*Lee Ann Brown*

Lee Ann Brown

November 14, 2001

Date of Mailing

**SUPPLEMENTAL PRELIMINARY AMENDMENT**

Commissioner for Patents  
Washington, D.C. 20231

Sir:

Prior to examination of the above-identified national phase patent application, please amend the application, as follows:

**In the Specification**

Please insert on page 1 between the title of the application and the first paragraph the following new paragraph:



#### CROSS-REFERENCE TO RELATED APPLICATIONS

This application is filed under the provisions of 35 U. S.C. §371 and claims the priority of International Patent Application No. PCT/DE00/00079 filed January 11, 2000, and which in turn claims priority of German Patent Application No. 199 00 635.0 filed January 11, 1999.

#### REMARKS

This claim to priority is being filed before the above-identified application meets all requirements under 35 U.S.C. §371(b).

Respectfully submitted,

A handwritten signature in cursive script, appearing to read "Marianne Fuierer".

Marianne Fuierer  
Registration No. 39,983  
Attorney for Applicants

INTELLECTUAL PROPERTY/  
TECHNOLOGY LAW  
P. O. Box 14329  
Research Triangle Park, NC 27709  
Phone: (919) 419-9350  
Fax: (919) 419-9354  
Attorney File: 4121-126

4121-126  
PATENT APPLICATION

---

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

---

**In re Application of:** BREITLING, et al.  
**Application No.:** New U.S. National Stage Application of  
PCT International Application No.  
PCT/DE00/00079  
**International Filing Date:** 11 January 2000  
**Priority Date Claimed:** 11 January 1999 (German Appl. No. 199 00  
635.0)  
**U.S. National Phase Filing Date:** Date of mailing identified below  
**Title:** SELECTION OF MONOCLONAL  
ANTIBODIES


---

**EXPRESS MAIL CERTIFICATE**

I hereby certify that I am mailing the attached documents to the  
Commissioner for Patents on the date specified, in an envelope  
addressed to the Commissioner for Patents, Box Patent Application,  
Washington, DC 20231, and Express Mailed under the provisions of  
37 CFR 1.10

Blake Crouch

Name of Person Mailing This Document

  
Signature

July 10, 2001

Date

EL666414295US

Express Mail Label Number

---

**PRELIMINARY AMENDMENT**

---

Commissioner for Patents  
BOX PATENT APPLICATION  
Washington, D.C. 20231

Sir:

09889182-01002

Prior to examination of the above-identified new national phase patent application, please amend the application, as follows:

**In the Specification**

On the bottom of page 4 and top of page 5, please replace the paragraph with the following paragraph:

Preferred antibody binding proteins are shown in figures 1 to 3. The antibody binding protein of figure 1 comprises the signal peptide of a mouse MHC class I k(k) molecule, four antibody binding domains of the L protein and the transmembrane domain of CD52. The DNA (SEQ ID NO: 3 from nucleotide 682-1782) and amino acid sequences (SEQ ID NO: 4) of the antibody binding protein are given between nucleotide numbers 682-1782. The antibody binding protein of figure 2 comprises the signal peptide of a mouse Ig kappa chain, two antibody binding sites of the G protein and the transmembrane domain of CD52. The DNA (SEQ ID NO: 1 from nucleotide 737-1420) and amino acid sequences (SEQ ID NO: 2) of the antibody binding protein are indicated between nucleotide numbers 737-1420. The antibody binding protein of figure 3 comprises the signal peptide of the mouse MHC class I k(k) molecule, two antibodies binding sites of the G protein and the transmembrane domain of PDGFR. The DNA (SEQ ID NO: 5 from nucleotide 682-1431) and amino acid sequences (SEQ ID NO: 6) of the antibody binding protein are given between nucleotide numbers 682-1431. The antibody binding sites of all three antibody binding proteins have, on a DNA level, codons which are optimized for expression in mammalian cells.

**In the Claims**

Please amend claims 1-20 to read as follows:

1. A method of selecting monoclonal antibodies, comprising the fusion of B lymphocytes with myeloma cells to form antibody-producing hybridoma cells, the antibodies being presented on the cell surface of the hybridoma cells by means of an antibody binding protein, and the binding of the antibodies to

antigens, wherein the antibody binding proteins are inserted in the hybridoma cells via the myeloma cells or in the hybridoma cells via the expression vectors coding therefor.

2. The method according to claim 1, wherein the antibody binding protein comprises a signal peptide, an antibody binding site independent of the antibody specificity and a membrane anchor.
3. The method according to claim 2, wherein the antibody binding protein comprises an Fc binding protein or portions thereof.
4. The method according to claim 2, wherein the antibody binding protein comprises a combination of Fc binding proteins or portions thereof.
5. The method according to claim 4, wherein the Fc binding protein is selected from the group consisting of CD16, CD32 and CD64.
6. The method according to claim 2, wherein the antibody binding protein comprises an antibody binding domain of proteins selected from the group consisting of A, G, L and LG.
7. The method according to claim 2, wherein the antibody binding protein comprises a combination of a signal peptide selected from the group consisting of a signal peptide of a mouse Ig cappa chain, and a signal peptide of a mouse MHC-class I k(k) molecule; an antibody binding site of a protein selected from the group consisting of protein A, G, L, and LG; and a transmembrane domain selected from the group consisting of PDGFR and CD52.
8. The method according to claim 7, wherein the antibody binding protein is selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 4 and SEQ ID NO: 6.



9. The method according to claim 1, wherein the hybridoma cells (over)express Rag1 and/or Rag2.
10. The method according to claim 1, wherein the antigens originate from an antigen library.
11. The method according to claim 1, wherein the antigens are bound to a carrier.
12. The method according to claim 11, wherein the carrier comprises magnetobeads.
13. The method according to claim 7, wherein the antigens comprise a fluorescence or biotin labeling.
14. The method according to claim 13, wherein the fluorescence labeling comprises FITC, TRITC, Cy3, Cy5, Cy5.5, Cy7 and phycoerythrin.
15. An antibody binding protein, wherein the antibody binding protein comprises a combination of the signal peptide selected from the group consisting of a mouse Ig cappa chain and a mouse MHC-class I k(k) molecule, an antibody binding site of proteins selected from the group consisting of A, G, L and LG, and a transmembrane domain selected from the group consisting of PDGFR and CD52.
16. The antibody binding protein according to claim 15, wherein the antibody binding protein comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 2, SEQ ID.NO 4 and SEQ ID NO:6 or an amino acid sequence differing therefrom by one or more amino acids.
17. DNA coding for the antibody binding protein according to claim 16, comprising:
  - (a) the DNA of an antibody binding protein selected from the group consisting of SEQ ID NO: 1 from nucleotide 737-1420, SEQ ID NO: 3 from nucleotide 682-1782, and SEQ ID NO: 5 from nucleotide 682-1431

[of figure 1, 2 or 3], a DNA differing therefrom by one or more base pairs, or

(b) a DNA related to the DNA of (a) via the degenerated code.

18. An expression vector, coding for the DNA according to claim 17.
19. Cells containing the expression vector according to claim 18.
20. An antibody directed against the antibody binding protein according to claim 16.

20071012168860

**REMARKS**

A marked-up version of amended paragraph in the specification and amended claims 1-20 are included herewith in Appendix A.

It is requested that the examination and prosecution of this application proceed on the basis of the English translation of the PCT International application included herewith and these amended claims 1-20.

Respectfully submitted,



Marianne Fuierer  
Registration No. 39,983  
Attorney for Applicants

INTELLECTUAL PROPERTY/  
TECHNOLOGY LAW  
P. O. Box 14329  
Research Triangle Park, NC 27709  
Phone: (919) 419-9350  
Fax: (919) 419-9354  
Attorney File: 4121-126

## APPENDIX A

### In the Specification

On the bottom of page 4 and top of page 5, replace the paragraph with the following paragraph:

Preferred antibody binding proteins are shown in figures 1 to 3. The antibody binding protein of figure 1 comprises the signal peptide of a mouse MHC class I k(k) molecule, four antibody binding domains of the L protein and the transmembrane domain of CD52. The DNA (SEQ ID NO: 3 from nucleotide 682-1782) and amino acid sequences (SEQ ID. NO: 4) of the antibody binding protein are given between nucleotide numbers 682-1782. The antibody binding protein of figure 2 comprises the signal peptide of a mouse Ig kappa chain, two antibody binding sites of the G protein and the transmembrane domain of CD52. The DNA (SEQ ID NO: 1 from nucleotide 737-1420) and amino acid sequences (SEQ ID NO: 2) of the antibody binding protein are indicated between nucleotide numbers 737-1420. The antibody binding protein of figure 3 comprises the signal peptide of the mouse MHC class I k(k) molecule, two antibodies binding sites of the G protein and the transmembrane domain of PDGFR. The DNA (SEQ ID NO: 5 from nucleotide 682-1431) and amino acid sequences (SEQ ID NO: 6) of the antibody binding protein are given between nucleotide numbers 682-1431. The antibody binding sites of all three antibody binding proteins have, on a DNA level, codons which are optimized for expression in mammalian cells.

### In the Claims

5. The method according to claim [3 or] 4, wherein the Fc binding protein is selected from the group consisting of CD16, CD32 [or] and CD64.
6. The method according to claim 2 [any of claims 2 to 5], wherein the antibody binding protein comprises an antibody binding domain of proteins selected from the group consisting of A, G, L and [or] LG.

7. The method according to claim 2, wherein the antibody binding protein comprises a combination of a [the] signal peptide selected from the group consisting of a signal peptide of a mouse Ig cappa chain, and a signal peptide of a mouse MHC-class I k(k) molecule; an antibody binding site of a protein[s] selected from the group consisting of protein A, G, L, and [or] LG; and [the] a transmembrane domain selected from the group consisting of PDGFR [or] and CD52.
8. The method according to claim 7, wherein the antibody binding protein is selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 4 and SEQ ID NO: 6. [that of figure 1, figure 2 or figure 3.]
9. The method according to claim 1 [any of claims 1 to 8], wherein the hybridoma cells (over)express Rag1 and/or Rag2.
10. The method according to claim 1 [any of claims 1 to 9], wherein the antigens originate from an antigen library.
11. The method according to claim 1 [any of claims 1 to 10], wherein the antigens are bound to a carrier.
13. The method according to claim 7, [any of claims 1 to 10], wherein the antigens comprise a fluorescence or biotin labeling.
15. An antibody binding protein, wherein the antibody binding protein comprises a combination of the signal peptide selected from the group consisting of a mouse Ig cappa chain [or] and a mouse MHC-class I k(k) molecule, an antibody binding site of proteins selected from the group consisting of A, G, L [or] and LG and a [the] transmembrane domain selected from the group consisting of PDGFR [or] and CD52.

16. The antibody binding protein according to claim 15, wherein the antibody binding protein comprises an [the] amino acid sequence selected from the group consisting of [figure 1, figure 2 or figure 3] SEQ ID NO: 2, SEQ ID.NO 4 and SEQ ID NO:6 or an amino acid sequence differing therefrom by one or more amino acids.
17. DNA coding for the antibody binding protein according to claim 16, comprising:
- (a) the DNA of an antibody binding protein selected from the group consisting of SEQ ID NO: 1 from nucleotide 737-1420, SEQ ID NO: 3 from nucleotide 682-1782, and SEQ ID NO: 5 from nucleotide 682-1431 [of figure 1, 2 or 3], a DNA differing therefrom by one or more base pairs, or
  - (b) a DNA related to the DNA of (a) via the degenerated code.

18/PR 75

09/889182  
JC18 Rec'd PCT/PTO 10 JUL 2001

### Selection of Monoclonal Antibodies

The present invention relates to a method of selecting monoclonal antibodies and to means which can be used therefor.

The production of monoclonal antibodies is based on a method developed by Kohler and Milstein. According to this method B lymphocytes are fused with myeloma cells so as to obtain antibody-producing hybridoma cells. Such a method comprises major drawbacks. In particular, it is time-consuming and expensive to select antibodies, since this calls for separate culturing of hybridoma cells. Due to the latter only a limited number of hybridoma cells is detected and thus not all of the antibodies can be selected, this being a drawback in particular when antibodies with maximum affinity for an antigen shall be selected.

It is thus the object of the present invention to provide a product by which monoclonal antibodies can be produced, the above drawbacks being avoided.

According to the invention this is achieved by the subject matters defined in the claims.

The present invention is based on Applicant's insights that monoclonal antibodies on the cell surface of hybridoma cells can be presented by means of an antibody binding protein. He realized that monoclonal antibodies can be selected by this without hybridoma cells having to be cultured separately. He also realized that monoclonal antibodies can be selected

09889182-011002

with respect to a determined and many (un)determined antigens of an antigen library. Furthermore, he found that monoclonal antibodies can also be selected with respect to their affinity intensity for certain antigens.

According to the invention Applicant's insights are used to provide a method of selecting monoclonal antibodies. Such a method comprises fusing B lymphocytes with myeloma cells to form antibody-producing hybridoma cells, the antibodies being presented on the cell surface of the hybridoma cells by means of an antibody binding protein, and binding of the antibodies to antigens.

The expression "B lymphocytes" comprises B lymphocytes of any kind and origin. They may also concern precursors of B lymphocytes. The B lymphocytes may originate from animals, such as mice, rats, rabbits, etc., or humans. The B lymphocytes may also originate from a healthy or diseased organism. It is favorable for them to originate from an immunized organism. It is particularly favorable for the B lymphocytes to code for human antibodies or portions thereof. If B lymphocytes from animals are concerned, this can be achieved when the animals are transgenic for the human antibodies or portions thereof. Such animals can be produced by common methods, it being an obvious thing to introduce the genes for the human antibodies or the portions thereof into embryonal stem cells from which the animals are then generated. B lymphocytes and their precursors may be provided by common methods.

The expression "myeloma cells" comprises myeloma cells of any kind and origin. They may also concern precursors of myeloma cells. Furthermore, the myeloma cells may originate from animals, such as mice, rats, rabbits, etc., or humans.

09889182.01.1002



Preferred myeloma cells are descendents from the mouse strains P3K, P3-X63.Ag8, X63.Ag8.653, NSO/1, Sp2/O-Ag14 and FO, the rat strains Y3-Ag1.2.3, YB2/0 and IR9834, and the human strains U266, SK007 and Karpas 707. Myeloma cells and their precursors can be provided by common methods.

The expression "antibody-producing hybridoma cells" comprises cells which form by fusion of B lymphocytes and myeloma cells and produce antibodies. Corresponding reference is made to the statements on B lymphocytes and myeloma cells. Hybridoma cells may include animal and/or human nucleic acids and/or proteins. Hybridoma cells can be cultured by common methods. It may also be favorable for the hybridoma cells to (over)express recombinases, e.g. Rag1 or Rag2, and/or mutases. This can be achieved by transfection of the hybridoma cells with corresponding expression vectors. The person skilled in the art knows such expression vectors.

The term "fusion of B lymphocytes with myeloma cells" concerns any method by means of which these cells may be fused. A method is favorable in which the cells are fused via polyethylene glycol. Reference is made to the examples.

The term "binding of the antibodies to antigens" concerns any method by which the antibodies expressed on the cell surface of the hybridoma cells can bind to antigens. The antigens can be bound to carriers, e.g. magnetobeads. They can also be labeled, e.g. fluorescence-labeled. For example FITC, TRITC, Cy3, Cy5, Cy5.5, Cy7 and phycoerythrin offer themselves as fluorescence markers. The antigens may also be coupled to biotin. Bound antigens may be detected by common methods, e.g. FACS analysis whereby the corresponding

2007-01-01 09:00:00

antibodies are also detected. Reference is made to the examples.

The expression "antibody binding protein" comprises any protein which may bind an antibody and present it on the cell surface of hybridoma cells. In particular, the protein may have a signal peptide, an antibody-binding site independent of the specificity of the antibody and a membrane anchor. Examples of such a protein are natural Fc binding proteins, such as CD16, CD32 and CD64. The protein may comprise a combination of a signal peptide, an antibody binding site and a membrane anchor, which does not occur in nature. Such a combination may comprise portions of natural Fc binding proteins. Furthermore, as a signal peptide it may have one of a mouse Ig kappa chain or a mouse MHC-class I k(k) molecule, as a membrane anchor it may include a transmembrane domain of PDGRF or CD52 and as an antibody binding site it may comprise an antigen binding domain of a bacterial protein, such as protein A, protein G, protein L or protein LG. It may be favorable for the combination to comprise several signal peptides, antibody binding sites and/or membrane anchors. It may be particularly favorable for the antibody binding protein, in particular the antibody binding domain of the bacterial proteins, to have codons which are optimized for expression in mammalian cells. A person skilled in the art knows which codons are concerned here.

Preferred antibody binding proteins are shown in figures 1 to 3. The antibody binding protein of figure 1 comprises the signal peptide of a mouse MHC class I k(k) molecule, four antibody binding domains of the L protein and the transmembrane domain of CD52. The DNA and amino acid sequences of the antibody binding protein are given between

09889182-011002

nucleotide numbers 682-1782. The antibody binding protein of figure 2 comprises the signal peptide of a mouse Ig kappa chain, two antibody binding sites of the G protein and the transmembrane domain of CD52. The DNA and amino acid sequences of the antibody binding protein are indicated between nucleotide numbers 737-1420. The antibody binding protein of figure 3 comprises the signal peptide of the mouse MHC class I k(k) molecule, two antibodies binding sites of the G protein and the transmembrane domain of PDGFR. The DNA and amino acid sequences of the antibody binding protein are given between nucleotide numbers 682-1431. The antibody binding sites of all three antibody binding proteins have, on a DNA level, codons which are optimized for expression in mammalian cells.

An antibody binding protein of figures 1, 2 or 3 may have an amino acid sequence which differs from the amino acid sequence in figure 1, 2 or 3 by one or more amino acids. The differences may lie in additions, deletions, substitutions and/or inversions of individual amino acids. However, the DNA of this antibody binding protein hybridizes with the DNA indicated in figure 1, 2 or 3. The term "hybridizing" refers to hybridization under common conditions, in particular at 20°C below the melting point of the DNA. Furthermore, the antibody binding protein having the modified amino acid sequence comprises whole or partial functions which can be compared with those of the antibody binding protein of figure 1, 2 or 3.

Another subject matter of the present invention relates to a nucleic acid which codes for an above antibody binding protein. The nucleic acid may be an RNA or a DNA. Preferred is a DNA which comprises the following:

09889182.01002

- (a) the DNA of an antibody binding protein of figure 1, 2 or 3, a DNA differing therefrom by one or more base pairs, or
- (b) a DNA related to the DNA from (a) by the degenerated genetic code.

The term "a DNA differing by one or more base pairs" comprises any DNA coding for an antibody binding protein of figure 1, 2 or 3, which hybridizes with the DNA of figure 1, 2 or 3. The differences may lie in additions, deletions, substitutions and/or inversions of individual base pairs. As to the term "hybridizing" reference is made to the above explanations.

A DNA according to the invention may be present as such or in combination with any other DNA. In particular, a DNA according to the invention, which codes for an antibody binding protein, may be present in an expression vector. The person skilled in the art is familiar with examples thereof. In the case of an expression vector for *E. coli* these are e.g. pGEMEX, pUC derivatives, pGEX-2T, pET3b and pQE-8. For the expression in yeast, e.g. pY100 and Ycpad1 have to be mentioned while e.g. pKCR, pEFBOS, pCDM8 and pCEV4 have to be indicated for the expression in animal cells. The baculovirus expression vector pAcSGHisNT-A is particularly suitable for the expression in insect cells.

The person skilled in the art knows how to insert the DNA according to the invention in an expression vector. He also knows that this DNA can be inserted in combination with a DNA coding for another protein or peptide, so that the DNA according to the invention can be expressed in the form of a fusion protein.

0989182.0102

Preferred expression vectors which contain a DNA according to the invention are shown in figures 1 to 3. The expression vectors pSEX11L4, pSEX11G2\* and pSEX15G2 are concerned. They were deposited with the DSMZ (*Deutsche Sammlung für Mikroorganismen und Zellkulturen* [German-type collection of microorganisms and cell cultures]) on December 14, 1998. In particular, pSEX11L4 was deposited under DSM 12580, pSEX11G2\* was deposited under DSM 12581 and pSEX15G2 was deposited under DSM 12582.

The person skilled in the art is familiar with suitable cells to express a cDNA according to the invention, which is present in an expression vector. Examples of such cells comprise the *E. coli* strains XL-1 Blue, Top 10 F, HB101, DH5alpha, x1776, JM101, JM 109, BL21 and SG 13009, the yeast strain *Saccharomyces cerevisiae* and *Pichia pastoris*, the animal cells L, NIH 3T3, FM3A, CHO, COS, Vero, HeLa, myeloma and hybridoma cells as well as the insect cells sf9.

The person skilled in the art also knows conditions of culturing transformed or transfected cells. He is also familiar with methods of isolating and purifying the protein or fusion protein expressed by the cDNA according to the invention.

Another subject matter of the present invention relates to an antibody directed against an above protein or fusion protein. Such an antibody may be prepared by common methods. It may be polyclonal or monoclonal. For its preparation it is favorable to immunize animals - in particular rabbits or chickens for a polyclonal antibody and mice for a monoclonal antibody - with an above (fusion) protein or with fragments thereof. Further "boosters" of the animals can be effected

0989132 01002

with the same (fusion) protein or with fragments thereof. The polyclonal antibody may then be obtained from the animal serum or egg. For the preparation of the monoclonal antibody, animal spleen cells are fused with myeloma cells.

Another subject matter of the present invention is a kit. Such a kit comprises one or more of the following components:

- (a) a DNA according to the invention,
- (b) a cell expressing a DNA according to the invention,
- (c) an antibody binding protein according to the invention,
- (d) an antibody according to the invention, and
- (e) common auxiliary substances such as carriers, buffers, solvents, controls, markers, detection reagents for components (a) - (d).

One or more representatives of the individual components may be present. As to the individual terms reference is made to the above statements. They apply here analogously.

The present invention distinguishes itself in that antibodies produced by hybridoma cells are presented on the cell surface of the hybridoma cells. This is done via an antibody binding protein. Such a protein may be introduced into the hybridoma cells via the myeloma cells used for the production of the hybridoma cells. The antibody binding protein may also be introduced into the hybridoma cells via an expression vector coding for it.

By means of the present invention it is possible to select antibodies. This can be done without much expenditure, since hybridoma cells do not have to be cultured separately. Complex mixtures of hybridoma cells can rather be used

09889182-011002



**Example 1: Preparation of myeloma cells which express an antibody binding protein on their cell surface**

**(A) Transient expression**

Cells of the myeloma cell line X63-Ag8.653 are used. These cells ( $10^7$ ) are transfected with 20-40  $\mu$ g of the expression vector SEX11G2\* according to the invention (cf. figure 2). Electroporation is carried out as transfection technique, which comprises two pulses of 2 ms at 500 V. The cells are incubated for 48 h at 37°C and 5-7.5 %  $\text{CO}_2$  in RPMI medium which contains 10 % FCS. Thereafter, the cells are washed with cold DPBS + 0.1 % Na azide before they are incubated for 45 minutes at 0°C with DPBS + 0.1 % Na azide plus 25  $\mu$ g/ml goat anti-calf antibody (FITC-labeled; GAB-FITC, Dianova company). Having been washed with DPBS + 0.1 % Na azide, the cells are incubated in DPBS + 0.1 % Na azide + 1  $\mu$ g/ml propidium iodide and subjected to FACS analysis following excitation with blue light.

It shows that the transfected myeloma cells have a green fluorescence which is due to the transient expression of an antibody binding protein on the cell surface of the myeloma cells.

**(B) Stable expression**

The myeloma cells obtained under (A) are subjected to G418 selection for 14-24 days before they are incubated using GAB-FITC and subjected to FACS analysis as described under (A). Myeloma cells which have a strong green fluorescence are subject to further G418 selection rounds or runs.

09889182.01002



The myeloma cell line X63-Ag8.653.3 is obtained which stably expresses an antibody binding protein on its cell surface.

**Example 2:      Production of hybridoma cells which express  
on their cell surface antibodies by means of  
an antibody binding protein**

**(A)**

10 Balb/c mice are immunized subcutaneously in each case with 100 µg killed *Helicobacter pylori* bacteria in complete Freund's adjuvant, which contains killed *Mycobacter tuberculosis* bacteria. After 4 or 7 weeks, an intraperitoneal booster injection with 100 µg killed *Helicobacter pylori*/*Mycobacter tuberculosis* bacteria is given. 100 µl blood serum are withdrawn from the mice before each immunization and after the last immunization, and the antigen-specific immune response of the mouse is tested in a Western blot. A degradation of bacterial whole protein of *Helicobacter pylori* and/or *Mycobacter tuberculosis* is used as antigen. The detection of bound mouse antibodies is made by an peroxidase-conjugated goat anti-mouse antibody (Dianova company). The spleen of mice having a marked antigen-specific immune response is removed and the lymphocytes are fused with cells of the myeloma cell line X63-Ag8.653.3 of Example 1 (B). The fusion is made by means of polyethylene glycol (cf. Goding, J.W., Cell Biology, Biochemistry and Immunology, 3<sup>rd</sup> edition (1996), Verlag Academic Press Limited, 24-28). Hybridoma cells are obtained. They are incubated in HAT medium at 37°C for 10 to 12 days. The hybridoma cell library 2A is obtained.

Hexapeptides with N-terminal biotin are synthesized. The peptides correspond to the 6C-terminal amino acids of 101 or 118 gene products of *Helicobacter pylori* or *Mycobacter*

09889182-011002

*tuberculosis*.  $10^3$  cells of the hybridoma cell library 2A are also washed with cold DPBS + 0.1 % Na azide and incubated for 45 minutes at 0°C with DPBS + 0.1 % Na azide + 10 µg/ml of the above biotin-labeled peptides. The cells are washed with cold DPBS + 0.1 % Na azide and incubated for 45 minutes at 0°C with 10 µg/ml streptavidine FITC. Having been washed with DPBS + 0.1 % Na azide, the cells are incubated in DPBS + 0.1 % Na azide + 1 µg/ml propidium iodide and subjected to FACS analysis after excitation with blue light.

It shows that the hybridoma cells have a green fluorescence. This fluorescence is due to the expression of antibodies on the cell surface of the hybridoma cells. Further studies show that the antibodies have an anti-*Helicobacter pylori* or *Mycobacter tuberculosis* activity.

#### (B)

Cells of the hybridoma cell line U98/6 which produce a mouse anti-urokinase antibody are used. These cells ( $10^7$ ) are transfected with 20-40 µg of the pSEX11G2\* expression vector according to the invention (cf. figure 2). Electroporation is carried out as a transfection technique, which comprises two pulses of 2 ms at 400 V. The cells are incubated for 48 h in incomplete AIM V-medium at 37°C and 5-7.5 %  $\text{CO}_2$ . Thereafter, the cells are washed with cold DPBS + 0.1 % Na azide before they are incubated at 0°C for 45 minutes with DPBS + 0.1 % Na azide + 10 µg/ml urokinase biotin. Having been washed with DPBS + 0.1 % Na azide, the cells are incubated in DPBS + 0.1 % Na azide + 10 µg/ml streptavidine FITC and subjected to FACS analysis after excitation with blue light.

It shows that the transfected hybridoma cells have a green fluorescence. This fluorescence is due to the expression of

The resulting hybridoma cells are subjected to G418 selection for 14 to 24 days before they are again incubated with urokinase-biotin and streptaividine-FICS and subjected to FACS analysis as described above. Hybridoma cells which have a strong green fluorescence are subjected to further G418 selection rounds.

Example 3: Selection of monoclonal antibodies which are expressed on the cell surface of hybridoma cells by means of an antibody binding protein

10<sup>3</sup> cells of the hybridoma cell line U98/6.3.3 of Example 2 (B) are mixed with 10<sup>7</sup> cells of the hybridoma cell line DOB.L1.3. The latter hybridoma cell line produces an antibody recognizing the C terminus of the human HLA-DO-β chain. It is expressed on the cell surface by means of an antibody binding protein the same as that in the hybridoma cell line U98/6.3.3 of Example 2(B). The cell mixture is washed with cold DPBS + 0.1 T Na azide and incubated at 0°C for 45 minutes with DPBS + 0.1 % Na azide + 10 µg/ml urokinase biotin. Having been washed with DPBS + 0.1 % Na azide, the cell mixture is incubated in DPBS + 0.1 % Na azide + 10 µg/ml streptavidine FITC and supplied to a FACS sorter following excitation with blue light.

Hybridoma cells with green fluorescence are selected. In further studies, they show an anti-urokinase activity. The hybridoma cell lines U98/6.3.3 S1-S50 are obtained.

**Example 4: Production and purification of an antibody binding protein according to the invention**

**(A)**

The DNA of figure 1 between nucleotide numbers 682-1782 is provided with BAMHI linkers, subsequently cleaved using BamHI, and inserted in the pQE-8 expression vector cleaved by BamHI (Qiagen company). The expression plasmid pQE-8/antibody binding protein is obtained. Such a plasmid codes for a fusion protein comprising 6 histidine residues (N terminus partner) and the antibody binding protein of fig. 1 according to the invention (C terminus partner). pQE-8/antibody binding protein is used for transforming *E. coli* SG 13009 (cf. Gottesman, S. et al., J. Bacteriol. 148, (1981), 265-273). The bacteria are cultured in an LB broth with 100 µg/ml ampicillin and 25 µg/ml kanamycin and induced with 60 µM isopropyl-β-D-thiogalactopyranoside (IPTG) for 4 h. Lysis of the bacteria is achieved by the addition of 6 M guanidine hydrochloride. Thereafter, chromatography (Ni-NTA resin) is carried out with the lysate in the presence of 8 M urea in accordance with the instructions from the manufacturer of the chromatography material (Qiagen company). The bound fusion protein is eluted in a buffer having a pH of 3.5. After its neutralization, the fusion protein is subjected to 18 % SDS polyacrylamide gel electrophoresis and stained with coomassie blue (cf. Thomas, J.O., and Kornberg, R.D., J. Mol. Biol. 149 (1975), 709-733).

09889182.01.002

It shows that an antibody binding protein (fusion protein) according to the invention can be prepared in highly pure form.

**(B)**

10<sup>8</sup> cells of the myeloma cell line X63-Ag8.653.3 obtained in Example 1 (B) are washed with PBS, taken up in PBS + 1 % Tween 20 and incubated on ice. Particulate cell components are separated by centrifugation at 30,000 g, and the supernatant is placed on an IgG sepharose column (IgG sepharose 6 Fast Flow Lab Pack from Pharmacia company). Unbound components are removed by washing and the antibody binding protein according to the invention is eluted in acidic pH.

Following its neutralization, the antibody binding protein is subjected to 18 % SDS polyacrylamide gel electrophoresis and stained using coomassie blue (see above).

It showed that an antibody binding protein (fusion protein) according to the invention can be obtained in highly pure form.

**Example 5: Preparation and detection of an antibody according to the invention**

A fusion protein of Example 4 according to the invention is subjected to 18 % SDS polyacrylamide gel electrophoresis. After staining the gel with 4 M sodium acetate, an about 41 kD band was excised from the gel and incubated in phosphate-buffered common salt solution. Gel pieces are sedimented before the protein concentration of the supernatant is determined by SDS polyacrylamide gel electrophoresis which

200710 28168860

is followed by coomassie blue staining. Animals are immunized with the gel-purified fusion protein as follows:

#### **Immunization protocol for polyclonal antibodies in rabbits**

35 µg of gel-purified fusion protein in 0.7 ml PBS and 0.7 ml of complete or incomplete Freund's adjuvant were used per immunization:

Day 0: 1<sup>st</sup> immunization (complete Freund's adjuvant)  
 Day 14: 2<sup>nd</sup> immunization (incomplete Freund's adjuvant; icFA)  
 Day 28: 3<sup>rd</sup> immunization (icFA)  
 Day 56: 4<sup>th</sup> immunization (icFA)  
 Day 80: bleeding to death.

The rabbit serum is tested in an immunoblot. For this purpose, a fusion protein of Example 4 according to the invention is subjected to SDS polyacrylamide gel electrophoresis and transferred to a nitrocellulose filter (cf. Khyse-Andersen, J., J. Biochem. Biophys. Meth. 10 (1984), 203-209). The Western blot analysis was carried out as described in Bock, C.-T. et al., Virus Genes 8, (1994), 215-229. For this purpose, the nitrocellulose filter is incubated with a first antibody at 37°C for one hour. This antibody is the rabbit serum (1:10000 in PBS). After several wash steps using PBS, the nitrocellulose filter is incubated with a second antibody. This antibody is an alkaline phosphatase-coupled monoclonal goat anti-rabbit IgG antibody (Dianova company) (1:5000) in PBS. 30 minutes of incubation at 37°C are followed by several wash steps using PBS and subsequently by the alkaline phosphatase detection reaction with developer solution (36 µM 5'-bromo-4-chloro-3-indolylphosphate, 400 µM nitro blue tetrazolium, 100 mM

0989182-011002

Tris-HCl, pH 9.5, 100 mM NaCl, 5 mM  $\text{MgCl}_2$ ) at room temperature until bands are visible.

It shows that polyclonal antibodies according to the invention can be prepared.

#### **Immunization protocol for polyclonal antibodies in chickens**

40  $\mu\text{g}$  of gel-purified fusion protein in 0.8 ml PBS and 0.8 ml of complete or incomplete Freund's adjuvant were used per immunization.

Day 0: 1<sup>st</sup> immunization (complete Freund's adjuvant)  
 Day 28: 2<sup>nd</sup> immunization (incomplete Freund's adjuvant; icFA)  
 Day 50: 3<sup>rd</sup> immunization (icFA)

Antibodies are extracted from egg yolk and tested in a Western blot. Polyclonal antibodies according to the invention are detected.

#### **Immunization protocol for monoclonal antibodies in mice**

12  $\mu\text{g}$  of gel-purified fusion protein in 0.25 ml PBS and 0.25 ml of complete or incomplete Freund's adjuvant are used per immunization. The fusion protein is dissolved in 0.5 ml (without adjuvant) in the 4<sup>th</sup> immunization.

Day 0: 1<sup>st</sup> immunization (complete Freund's adjuvant)  
 Day 28: 2<sup>nd</sup> immunization (incomplete Freund's adjuvant; icFA)  
 Day 56: 3<sup>rd</sup> immunization (icFA)  
 Day 84: 4<sup>th</sup> immunization (PBS)  
 Day 87: fusion.

20071028T090918

Supernatants of hybridomas are tested in a Western blot. Monoclonal antibodies according to the invention are detected.

20071022 21:00:00



### Amended Claims

1. A method of selecting monoclonal antibodies, comprising the fusion of B lymphocytes with myeloma cells to form antibody-producing hybridoma cells, the antibodies being presented on the cell surface of the hybridoma cells by means of an antibody binding protein, and the binding of the antibodies to antigens, wherein the antibody binding proteins are inserted in the hybridoma cells via the myeloma cells or in the hybridoma cells via the expression vectors coding therefor.
2. The method according to claim 1, wherein the antibody binding protein comprises a signal peptide, an antibody binding site independent of the antibody specificity and a membrane anchor.
3. The method according to claim 2, wherein the antibody binding protein comprises an Fc binding protein or portions thereof.
4. The method according to claim 2, wherein the antibody binding protein comprises a combination of Fc binding proteins or portions thereof.
5. The method according to claim 3 or 4, wherein the Fc binding protein is CD16, CD32 or CD64.
6. The method according to any of claims 2 to 5, wherein the antibody binding protein comprises an antibody binding domain of proteins A, G, L or LG.

2007-12-01 09:12:00

7. The method according to claim 2, wherein the antibody binding protein comprises a combination of the signal peptide of a mouse Ig cappa chain or a mouse MHC-class I k(k) molecule, an antibody binding site of proteins A, G, L or LG and the transmembrane domain of PDGFR or CD52.
8. The method according to claim 7, wherein the antibody binding protein is that of figure 1, figure 2 or figure 3.
9. The method according to any of claims 1 to 8, wherein the hybridoma cells (over)express Rag1 and/or Rag2.
10. The method according to any of claims 1 to 9, wherein the antigens originate from an antigen library.
11. The method according to any of claims 1 to 10, wherein the antigens are bound to a carrier.
12. The method according to claim 11, wherein the carrier comprises magnetobeads.
13. The method according to any of claims 1 to 10, wherein the antigens comprise a fluorescence or biotin labeling.
14. The method according to claim 13, wherein the fluorescence labeling comprises FITC, TRITC, Cy3, Cy5, Cy5.5, Cy7 and phycoerythrin.
15. An antibody binding protein, wherein the antibody binding protein comprises a combination of the signal peptide of a mouse Ig cappa chain or a mouse MHC-class

I k(k) molecule, an antibody binding site of proteins A, G, L or LG and the transmembrane domain of PDGFR or CD52.

16. The antibody binding protein according to claim 15, wherein the antibody binding protein comprises the amino acid sequence of figure 1, figure 2 or figure 3 or an amino acid sequence differing therefrom by one or more amino acids.
17. DNA coding for the antibody binding protein according to claim 16, comprising:
  - (a) the DNA of an antibody binding protein of figure 1, 2 or 3, a DNA differing therefrom by one or more base pairs, or
  - (b) a DNA related to the DNA of (a) via the degenerated code.
18. An expression vector, coding for the DNA according to claim 17.
19. Cells containing the expression vector according to claim 18.
20. An antibody directed against the antibody binding protein according to claim 16.

**Abstract of the Disclosure**

The present invention relates to a method of selecting monoclonal antibodies, comprising the fusion of B lymphocytes with myeloma cells to form antibody-producing hybridoma cells, wherein the antibodies are presented on the cell surface of the hybridoma cells by means of an antibody binding protein, and to the binding of the antibodies to antigens. The invention also concerns means usable for this purpose.

09889183-01002

09/889182

1/18

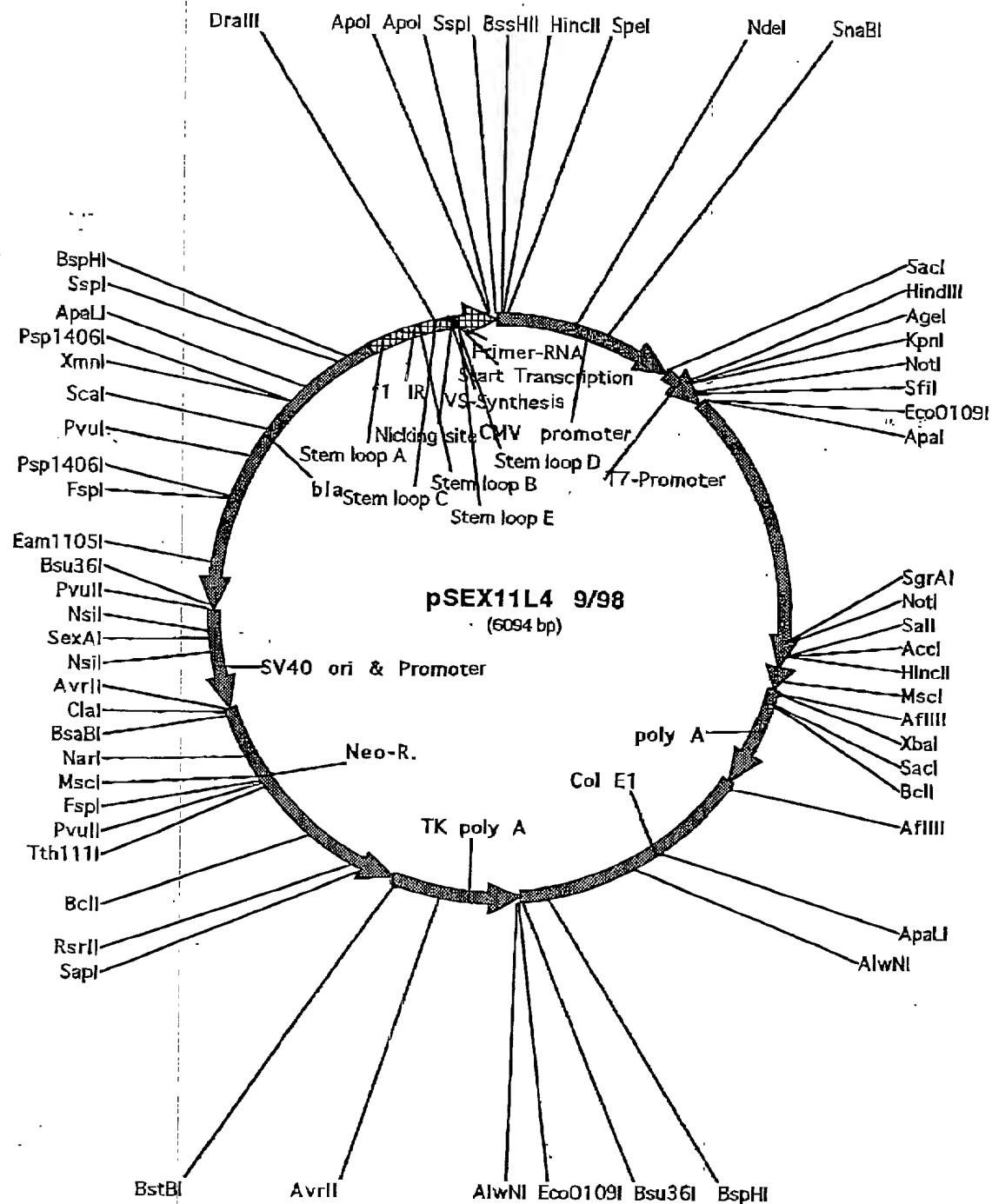


Fig. 1

09/889182

2/18

BstHII HincII SpeI  
 1 GCGCGCGTTGACATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTCATTA  
 60 GTTCATAGCCCATATATGGAGTTCGCGGTTACATAACTTACGGTAAATGGCCCGCCTGG  
 119 CTGACCGCCCAACGACCCCGCCATTGACGTCAATAATGACGTATGTTCCCATAGTAA  
 178 CCGCAATAGGGACTTTCCATTGACGTCAATGGGTGGACTATTTACGGTAAACTGCCAC  
 NdeI  
 237 TTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGG  
 CMV promoter  
 296 TAAATGGCCCGCTGGCATTATGCCAGTACATGACCTTATGGGACTTTCCTACTTGGC  
 SnaBI  
 355 AGTACATCTACGTATTAGTCATCGCTATTACCATGGTGATGCGGTTTTGGCAGTACATC  
 414 AATGGGCGTGGATAGCGGTTTGA CTACGGGGATTCCAAGTCTCCACCCCATGACGT  
 473 CAATGGGAGTTTGT TTTGGCACCAAAATCAACGGGACTTCCAAATGTCGTAACAAC  
 SacI  
 532 CCGCCCCATTGACGCAAAATGGGCGGTAGGCGGTACGGTGGGAGGTCTATATAAGCAGA  
 T7-Promoter  
 591 GGTCTCTGGCTAACTAGAGAACCCTGCTTACTGGCTTATCGAAATTAATACGACTCA  
 Agel  
 HindIII KpnI  
 650 CTATAGGGAGACCCCAAGCTTGGTACCGGTGCGATGGCACCCCTGCATGCTGCTCCTGCTG  
 1 MetAlaProCysMetLeuLeuLeuLeu  
 SfiI NotI Apal EcoO109I  
 709 TTGGCGCGCCCTGGCCCGACTCAGACCGCGCGGGGCCCCAAAGGAGAAGACCCC  
 10 LeuAlaAlaLeuAlaProThrGlnThrArgAlaGlyAlaGlnLysGluLysThrPr  
 768 CGAGGAGCCCAAGGAGGAGGTGACCATCAAGGCCAACCTGATCTACGCGGACGCAAGA  
 29 oGluGluProLysGluGluValThrIleLysAlaAsnLeuIleTyrAlaAspGlyLysT  
 827 CCCAGACCGCGAGTTCAAGGGCACCTTCGAGGAGGCCACCGCGGAGGCTACCGCTAC  
 49 hrGlnThrAlaGluPheLysGlyThrPheGluGluAlaThrAlaGluAlaTyrArgTyr  
 886 GCCGACGCCCTGAAGAAGGACAACGGCGAGTACACCGTGGACGTGGCCGACAAGGGCTA  
 69 AlaAspAlaLeuLysLysAspAsnGlyGluTyrThrValAspValAlaAspLysGlyTy  
 945 CACCTGAACATCAAGTTCGCCGCAAGGAGAAGACCCCGAGGAGCCCAAGGAGGAGG  
 88 rThrLeuAsnIleLysPheAlaGlyLysGluLysThrProGluGluProLysGluGluV  
 1004 TGACCATCAAGGCCAACCTGATCTACGCCGACGGCAAGACCCAGACCCCGAGTTCAAG  
 108 alThrIleLysAlaAsnLeuIleTyrAlaAspGlyLysThrGlnThrAlaGluPheLys  
 1063 GGCACCTTCGAGGAGGCCACCGCGGAGGCTACCGCTACGCCGACGCCCTGAAGAAGGA  
 128 GlyThrPheGluGluAlaThrAlaGluAlaTyrArgTyrAlaAspAlaLeuLysLysAs  
 1122 CAACGGCGAGTACACCGTGGACGTGGCCGACAAGGGCTACACCTGAACATCAAGTTTCG  
 147 pAsnGlyGluTyrThrValAspValAlaAspLysGlyTyrThrLeuAsnIleLysPheA  
 1181 CCGGCAAGGAGAAGACCCCGAGGAGCCCAAGGAGGAGTGACCATCAAGGCCAACCTG  
 167 lAlaGlyLysGluLysThrProGluGluProLysGluGluValThrIleLysAlaAsnLeu  
 1240 ATCTACGCCGACGGCAAGACCCAGACCGCGGAGTTCAAGGGCACCTTCGAGGAGGCCAC  
 187 lIleTyrAlaAspGlyLysThrGlnThrAlaGluPheLysGlyThrPheGluGluAlaTh  
 1299 CGCGGAGGCTACCGCTACCGGACGCGCTGAAGAAGGACAACGGCGAGTACCGCTGG  
 206 rAlaGluAlaTyrArgTyrAlaAspAlaLeuLysLysAspAsnGlyGluTyrThrValA  
 1358 ACGTGGCCGACAAGGCTACACCTGAACATCAAGTTCGCCGCAAGGAGAAGACCCCC  
 226 spValAlaAspLysGlyTyrThrLeuAsnIleLysPheAlaGlyLysGluLysThrPro

Fig. 1 (cont'd I)

09/ 889182

3/18

1417 GAGGAGCCCAAGGAGGAGGTGACCATCAAGGCCAACCTGATCTACGCCAGCGGCAAGAC  
246> GluGluProLysGluGluVal Thr I l eLysAlaAsnLeuI l eTyrAlaAspGlyLysTh  
1476 CGAGACCGCCGAGTTCAAGGGCACCTTCGAGGAGGCCACCGCGGAGGCCTACCGCTACG  
265> rGlnThrAlaGluPheLysGlyThrPheGluGluAlaThrAlaGluAlaTyrArgTyrA  
1535 CCGACGCCCTGAAGAAGGACAACGGCGAGTACACCTGGACGTGGCCGACAAGGGCTAC  
285> lAspAlaLeuLysLysAspAsnGlyGluTyr Thr ValAspValAlaAspLysGlyTyr  
SgrAI NotI  
1594 ACCCTGAACATCAAGTTTCGCCGGCGCGGCCGAGAACAAAACTCATCTCAGAAGAGGA  
305> ThrLeuAsnI l eLysPheAlaGlyAlaAlaAlaGluGlnLysLeuI l eSerGluGluAs  
Sall  
HincII  
AccI  
1653 TCTGAATGGGGCCGTCGACGGACAAAACGACACCAGCCAAACGACAGCCCTCAGCAT  
324> pLeuAsnGlyAlaValAspGlyGlnAsnAspThrSerGlnThrSerSerProSerAlaS  
MscI  
1712 CCAGCAACATAAGCGGAGGCATTTTCTTTTCTTCGTGGCAATGCCATAATCCACCTC  
344> erSerAsnI l eSerGlyGlyl l ePheLeuPhePheValAlaAsnAlaI l eI l eHisLeu  
AflIII XbaI  
1771 TTCTGCTTCAGTTGAGGTGACACGTCTAGAGCTATTCTATAGTGTCACCTAAATGCTAG  
364> PheCysPheSer \*\*\*  
BclI  
1830 AGCTCGCTGATCAGCCTCGACTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTGCCCT  
poly A  
1889 CCCCCGTGCCTTCCTTGACCCTGGAAGGTGCCACTCCCCTGTCCTTCTAATAAAAT  
1948 GAGGAAATTGCATCGCATTGTCTGAGTAGGTGTCATTCTATTCTGGGGGTGGGGTGGG  
2007 GCAGGACAGCAAGGGGGAGGATTGGGAAGACAATAGCAGGCATGCTGGGGATGCGGTGG  
2066 GCTCTATGGCTTCTGAGGCGGAAAGAACCAGTGCGGGTAATACGGTTATCCACAGAATC  
AflIII  
2125 AGGGGATAACGCAGGAAAGAATCTGTAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTA  
2184 AAAAGGGCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCTGACGAGCATCACAAA  
2243 AATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAGATAACGAGCGTT  
2302 TCCCCCTGGAAGCTCCCTCGTGCCTCTCCTGTTCCGACCTGCCGCTTACCGGATACC  
2361 TGTCGCTTTTCTCCCTTCGGGAAGCGTGGCGCTTCTCATAGCTCACGCTGTAGGTAT  
ApaI  
2420 CTCAGTTCGGTGTAGGTGCTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCGTTCA  
Col E1  
2479 GCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACAGG  
AlwNI  
2538 ACTTATCGCCACTGGCAGCAGCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGC  
2597 GGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGGACAGTATT  
2656 TGGTATCTCGCTCTGCTGAAGCCAGTTACCTTCGGAAGAGAGTTGGTAGCTCTTGAT  
2715 CCGGCAAAACAAACCCGCTGGTAGCGGTGGTTTTTTTGTGTTTGAAGCAGCAGATTACG  
2774 CGCAAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCA

Fig. 1 (cont'd II)

09/889182

4/18

2833 GTGGAACGAACTCACGTTAAGGGATTTGGTCATGAGATTATCAAAAAGGATCTTCA  
2892 CCTAGATCCTTTTAAATTAAAAATGAAGTTTTAAATCAATCTAAAGTATATATGAGTAA

EcoO109I  
Bsu36I AlwNI  
2951 CCTGAGGCTATGGCAGGGCTGCCGCCCGACGTTGGCTGCGAGCCCTGGGCCCTTACC

3010 CGAACTTGGGGGTGGGTGGGGAAAAGGAAGAAACGCGGGCGTATTGGCCCAATGGG  
3069 GTCTCGGTGGGTATCGACAGAGTGCCAGCCCTGGGACCGAACCCCGCTTATGAACA

TK poly A  
3128 AACGACCAACACCGTGCGTTTTATTCTGTCTTTTATTGCCGTCATAGCGGGGTTC

AvrII  
3187 TTCCGGTATTGTCTCCTTCCGTGTTTCAGTTAGCCTCCCCCTAGGGTGGGCGAAGAACT

3246 CCAGCATGAGATCCCCGCTGGAGGATCATCCAGCCGGCGTCCCGGAAAACGATTCCG  
3305 AAGCCCAACCTTTCATAGAAGCGCGGTGGAATCGAAATCTCGTGATGGCAGGTTGGG

BstBI  
3364 CGTCGCTTGGTCGGTCATTTGGAACCCAGAGTCCCGCTCAGAAAGACTCGTCAAGAAG  
2634...PhePheGluAspLeuLeu  
3423 GCGATAGAAGCGGATGCGCTGCGAATCGGGAGCGGCGATACCGTAAAGCAGGAGGAGC  
2564ArgTyrPheAlaIleArgGlnSerAspProAlaAlaIleGlyTyrLeuValLeuPheAr

SapI  
3482 GGTGAGCCCATTCGCCGCCAAGCTCTTCAGCAATATCAGGGTAGCCAACGCTATGTCC  
2364gAspAlaTrpGluGlyGluLeuGluAlaIleAspArgThrAlaLeuAlaIleAspG

RsrII  
3541 TGATAGCGGTCCGCCACCCAGCCGGCCACAGTCGATGAATCCAGAAAAGCGGCCATT  
2164InTyrArgAspAlaValGlyLeuArgGlyCysAspIlePheGlySerPheArgGlyAsn  
3600 TTCACCATGATATTCGCAAGCAGGCATCGCCATGGGTACGACGAGATCCTCGCCGT  
1974GluValMetIleAsnProLeuCysAlaAspGlyHisThrValValLeuAspGluGlyAs  
3659 CGGGCATGCTCGCCTTGAGCCTGGCGAACAGTTCGGCTGGCGGAGCCCTGATGCTCT  
1774pProMetSerAlaLysLeuArgAlaPheLeuGluAlaProAlaLeuGlyGlnHisGluG

BclI  
3718 TGATCATCTGATCGACAAGACCGGCTTCCATCCGAGTACGTGCTCGCTCGATGCGATG  
1574InAspAspGlnAspValLeuGlyAlaGluMetArgThrArgAlaArgGluIleArgHis  
3777 TTTGCTTGGTGGTCCAATGGGAGGTAGCCGGATCAAGCGTATGACGCGCGCCGATTG  
1384LysAlaGlnHisAspPheProCysThrAlaProAspLeuThrHisLeuArgArgMetAl  
3836 CATGACCATGATGGATCTTTCTCGGAGGAGCAAGGTGAGATGACAGGAGATCCTGC  
1184aAspAlaMetIleSerValLysGluAlaProAlaLeuHisSerSerLeuLeuAspGlnG

Tth111I Pvull  
3895 CCCGGCACTTCGCCCAATAGCAGCCAGTCCCTTCCCGCTTCAGTGACAACTCGAGCAC  
984IyProValGluGlyLeuLeuLeuTrpAspArgGlyAlaGluThrValValAspLeuVal

Neo-R.  
FspI MscI  
3954 AGCTGCGCAAGGAACGCCCGCTCGTGGCCAGCCAGATAGCCGCGTGCCTCGTCTTGCA  
794AlaAlaCysProValGlyThrThrAlaLeuTrpSerLeuArgAlaAlaGluAspGlnLe

NarI  
4013 GTTCATTAGGGCACCGGACAGGTGCGTCTTGACAAAAGAACCGGGCGCCCTGCGCT  
594uGluAsnLeuAlaGlySerLeuAspThrLysValPheLeuValProArgGlyGlnAlaS  
4072 GACAGCCGGAACACGGCGGCATCAGAGCAGCCGATTGTCTGTGTGCCAGTCATAGCC  
394erLeuArgPheValAlaAlaAspSerCysGlyIleThrGlnGlnAlaTrpAspTyrGly  
4131 GAATAGCTCTCCACCAAGCGGCGGAGAACCTGCGTGCAATCCATCTTGTTCATCA  
204PheLeuArgGluValTrpAlaAlaProSerGlyAlaHisLeuGlyAspGlnGluIleMe

BsaBI ClaI AvrII  
4190 TCGGAAACGATECTCATCTGTCTCTTGATCGATCTTGC AAAAGCCTAGGCCTCCAAA  
04t  
4249 AAAGCCTCCTCACTCTCTGGAATAGCTCAGAGGCCGAGGAGGCGGCCTCGGCCTCG

4308 CATAAATAAAAAAATTAGTCAGCCATGGGGCGGAGAATGGGCGGAACCTGGGCGGAGTT

Fig. 1 (cont'd III)



09/889182

5/18

SV40 ori & Promoter NsiI  
4367 AGGGGCGGGATGGGCGGAGTTAGGGGCGGGACTATGGTTGCTGACTAATTGAGATGCAT

SexAI  
4426 GCTTTGCATACCTTCTGCCTGCTGGGGAGCCTGGGGACTTTCCACACCTGGTTGCTGACT

NsiI  
4485 AATTGAGATGCATGCTTTGCATACCTTCTGCCTGCTGGGGAGCCTGGGGACTTTCCACAC

PvuII Bsu36I  
4544 CCTAACTGACACACATTCACAGCTGGTTCTTCCGCCTCAGGACTCTTCCTTTTCAA

4603 TAAATCAATCTAAAGTATATATGAGTAAACTTGGTCTGACAGTTACCAATGCTTAATCA  
2874 •••TrpHisLysIleLeu  
Eam1105I  
4662 GTGAGGCACCTATCTCAGCGATCTGTCTATTTCTGTTTCATCCATAGTTGCCTGACTCCCC  
2814 uSerAlaGlyIleGluAlaIleGlnArgAsnArgGluAspMetThrAlaGlnSerGlyT  
4721 GTCGTGTAGATAACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCAATGAT  
2614 hrThrTyrIleValValIleArgSerProLysGlyAspProGlyLeuAlaAlaIleIle  
4780 ACCGCGAGACCCACGCTCACC GGCTCCAGATTATCAGCAATAAACCCAGCCAGCCGGAA  
2424 GlyArgSerGlyArgGluGlyAlaGlySerLysAspAlaIlePheTrpGlyAlaProLeu  
4839 GGGCCGAGCGCAGAAGTGGTCTGCAACTTTATCCGCCTCCATCCAGTCTATTAATTGT  
2224 uAlaSerArgLeuLeuProGlyAlaValLysAspAlaGluMetTrpAspIleLeuGlnG  
FspI Psp1406I  
4898 TGCCGGGAAGCTAGAGTAAGTAGTTCGCCAGTTAATAGTTTGC GCAACGTTGTTGCCAT  
2024 InArgSerAlaLeuThrLeuLeuGluGlyThrLeuLeuLysArgLeuThrThrAlaMet  
4957 TGCTACAGGCATCGTGGTGTACGCTCGTCGTTTGGTATGGCTTCATTACGCTCCGGTT  
1834 AlaValProMetThrThrAspArgGluAspAsnProIleAlaGluAsnLeuGluProGlu  
5016 CCCAACGATCAAGGCGAGTTACATGATCCCCCATGTTGTGCAAAAAGCGGTTAGCTCC  
1634 uTrpArgAspLeuArgThrValHisAspGlyMetAsnHisLeuPheAlaThrLeuGluL  
PvuI  
5075 TTCGGTCTCCGATCGTTGTGAGAAGTAAGTTGGCCGAGTGTTATCACTCATGGTTAT  
1434 yProGlyGlyIleThrThrLeuLeuLeuAsnAlaAlaThrAsnAspSerMetThrIle  
bIa  
5134 GGCAGCACTGCATAATTCTCTTACTGTGATGCCATCCGTAAGATGCTTTTCTGTGACTG  
1244 AlaAlaSerCysLeuGluArgValThrMetGlyAspThrLeuHisLysGluThrValPr  
ScaI  
5193 GTGAGTACTCAACCAAGTCATTCTGAGAATAGTGTATGCGGCGACCGAGTTGCTCTTGC  
1044 oSerTyrGluValLeuAspAsnGlnSerTyrHisIleArgArgGlyLeuGlnGluGlnG  
5252 CCGCGCTCAATACGGGATAATACCGGCCACATAGCAGAACTTTAAAGTGCTCATCAT  
844 IyAlaAspIleArgSerLeuValAlaGlyCysLeuLeuValLysPheThrSerMetMet  
Psp1406I  
XmnI  
5311 TGGAAAACGTTCTTCGGGGCGAAAACCTCTCAAGGATCTTACCGCTGTTGAGATCCAGTT  
654 ProPheArgGluGluProArgPheSerGluLeuIleLysGlySerAsnLeuAspLeuGlu  
ApaI  
5370 CGATGTAAACCACTCGTGACCCCACTGATCTTCAGCATCTTTTACTTTACCAGCGTT  
454 uIleTyrGlyValArgAlaGlyLeuGlnAspGluAlaAspLysValLysValLeuThrG  
5429 TCTGGGTGAGCAAAAACAGGAAGGCAAAATGCCGCAAAAAGGGAATAAGGCGACACG  
254 IuProHisAlaPheValProLeuCysPheAlaAlaPhePheProIleLeuAlaValArg  
SspI  
5488 GAAATGTTGAATACTCATACTCTTCCCTTTTCAATATTATTGAAGCATTTATCAGGGTT  
64 PheHisGlnIleSerMet  
BspHI  
5547 ATTGTCTCATGAGCGGATACATATTTGAATGTATTTAGAAAAATAACAAATAGGGGTT  
5606 CGCGGCACATTTCCCCGAAAAGTGCCACCTGACGCGCCCTGTAGCGGCGCATTAAGCGC

Stem loop A  
5665 GCGGGGTGTGGTGGTTACGCGCAGCGTGACCGCTACACTTGCCAGCGCCCTAGCGCCCG

Fig. (cont'd IV)

09/889182

6/18

5724 CTCTTTTCGCTTTCTTCCCTTCTTTCTCGCCACGTTCCGCGGCTTTCCCCGTCAAGCT

5783 CTAATCGGGGGCTCCCTTTAGGGTTCCGATTAGTGCTTTACGGCACCTCGACCCAA

f1 IR                      Stem loop B

5842 AAACTTGATTAGGGTGATGGTTCACGTAGTGGGCCATCGCCCTGATAGACGGTTTTTC

DraIII                      Stem loop C                      Primer-RNA

5901 GCCCTTTGACGTTGGAGTCCACGTTCTTTAATAGTGGACTCTTGTTCCAAACTGGAACA

Start Transcription  
VS-Synthesis                      Nicking site                      Stem loop D                      Stem loop E

5960 AGACTCAACCTATCTCGGTCTATTCTTTTGATTATAAGGGATTTGCCGATTTCCGC

6019 CTATTGGTTAAAAATGAGCTGATTTAACAAAAATTTAACGCGAATTTAACAAAAATAT

ApoI                      ApoI                      SspI

6078 TAACGCTTACAATTAC

Fig. 1 (cont'd V)

0989182-01002

09/889182

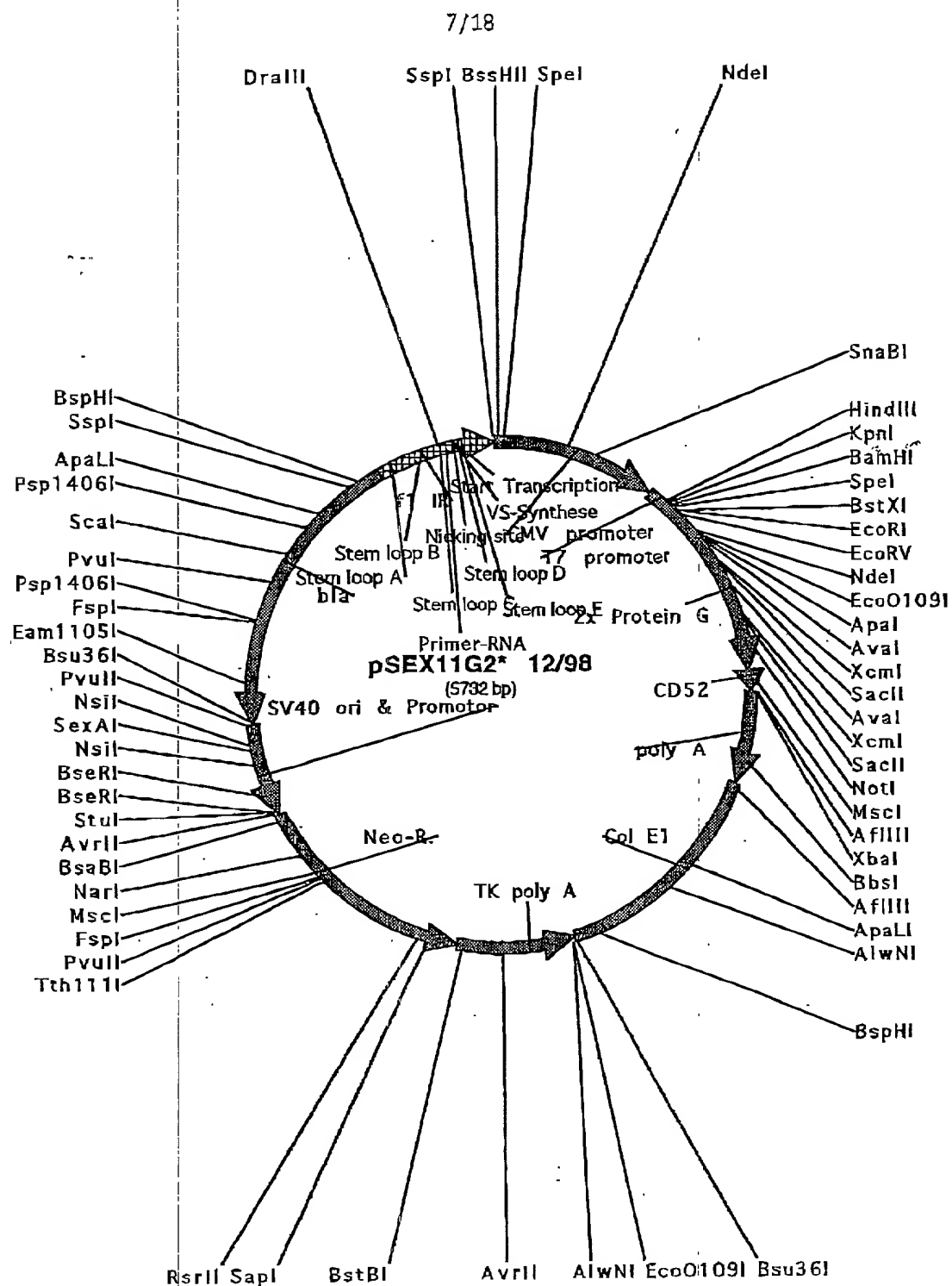


Fig. 2

09/889182

8/18

BssHII SpeI  
 1 GCGCGCGTTGACATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGGT  
 55 CATTAGTTCATAGCCCATATATGGAGTTCGCGTTACATAACTTACGGTAAATG  
 109 GCCCGCCTGGCTGACCGCCCAACGACCCCGCCCATTTGACGTCAATAATGACGT  
 163 ATGTTCCCATAGTAACGCCAATAGGGACTTTCATTGACGTCAATGGGTGGACT  
 NdeI  
 217 ATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGATCATATGCCAAGTA  
 CMV promoter  
 271 CGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCAGT  
 SnaBI  
 325 ACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCG  
 379 CTATTACCATGGTGATGCGGTTTTGGCAGTACATCAATGGGCGTGGATAGCGGT  
 433 TTGACTCACGGGGATTTCGAAGTCTCCACCCCATTTGACGTCAATGGGAGTTTGT  
 487 TTTGGCACAAAATCAACGGGACTTTCAAAATGTCGTAACTCCGCCCAT  
 541 TGACGCAAATGGGCGGTAGGCGGTGACGGTGGGAGGTCTATATAAGCAGAGCTC  
 T7 promoter  
 595 TCTGGCTAACTAGAGAACCCACTGCTTACTGGCTTATCGAAATTAATACGACTC  
 HindIII KpnI BamHI SpeI BstXI  
 649 ACTATAGGGAGACCAAGCTTGGTACCGAGCTCGGATCCACTAGTAACGGCCGC  
 EcoRI EcoRV  
 703 CAGTGTGCTGGAATTCGGCTTGGGGATATCCACCATGGAGACAGACACTCCT  
 1 Met Gl u Thr Asp Thr Leu Le  
 NdeI  
 757 GCTATGGGTACTGCTGCTCTGGGTTCAGGTTCCACTGGTGACTATCCATATGA  
 7 Leu Trp Val Leu Leu Leu Trp Val Pro Gly Ser Thr Gly Asp Tyr Pro Tyr As  
 ApaI  
 EcoO109I Aval  
 811 TGTTCCAGATTATGCTGGGGCCCAAGGCCGAGGTGATCGATGCCAGCGAGCT  
 25 pVal Pro Asp Tyr Ala Gly Ala Gl nLys Pro Gl uVal l l eAspAl aSer Gl uLe  
 865 GACCCCGCGCTGACCACCTACAAGCTAGTGATCAACGGCAAGACCCTGAAGGG  
 43 uThr ProAl aVal Thr Thr Tyr Lys LeuVal l l eAsnGlyLysThr LeuLysGl  
 XcmI SacII  
 919 CGAGACCACACCGAGGCGGTGGACGCCGCCACCGCGGAGAAGGTGTTCAAACA  
 61 yGl uThr Thr Thr Gl uAl aVal AspAl aAl aThrAl aGl uLysVal PheLysGl  
 973 ATACGCTAATGACAACGGGTGCGACGGCGAGTGGACTTACGACGACGCCACCAA  
 79 nTyrAl aAsnAspAsnGlyVal AspGly Gl uTrp Thr Tyr AspAspAl aThr Ly  
 Aval  
 2x Protein G  
 1027 GACCTTCACCGTGACCGAGAAGCCCGAGGTGATCGATGCCAGCGAGCTGACCCC  
 97 sThr PheThr Val Thr Gl uLysPro Gl uVal l l eAspAl aSer Gl uLeuThr Pr

Fig. 2 (cont'd I)

0989182-01002

09/889182

9/18

1081 CGCCGTGACCACCTACAAGCTAGTGATCAACGGCAAGACCCTGAAGGGCGAGAC  
 115 ▶ aAlaValThrThrTyrLysLeuValIleAsnGlyLysThrLeuLysGlyGluTh

XcmI SacII  
 1135 CACCACCGAGGCGGTGGACGCCGCCACCGCGGAGAAGGTGTTCAAACAATACGC  
 133 ▶ rThrThrGluAlaValAspAlaAlaThrAlaGluLysValPheLysGlnTyrAl  
 1189 TAATGACAACGGGTCGACGGCGAGTGGACTTACGACGACGCCACCAAGACCTT  
 151 ▶ aAsnAspAsnGlyValAspGlyGluTrpThrTyrAspAspAlaThrLysThrPh

NotI  
 1243 CACCGTGACCGAGGCGGCCGAGAACAAAACTCATCTCAGAAGAGGATCTGAA  
 169 ▶ eThrValThrGluAlaAlaAlaGluGlnLysLeuIleSerGluGluAspLeuAs

1297 TGGGGCCGTCGACGGACAAAACGACACCGCCAAACCAGCAGCCCTCAGCATC  
 187 ▶ nGlyAlaValAspGlyGlnAsnAspThrSerGlnThrSerSerProSerAlaSe

CD52 MscI  
 1351 CAGCAACATAAGCGGAGGCATTTTCCTTTCTTCGTGGCCAAATGCCATAATCCA  
 205 ▶ rSerAsnIleSerGlyGlyIlePheLeuPhePheValAlaAsnAlaIleIleHi

AflIII XbaI  
 1405 CCTCTTCTGCTTCAGTTGAGGTGACACGTCTAGAGCTATTCTATAGTGTCACCT  
 223 ▶ sLeuPheCysPheSer \*\*\* ←

1459 AAATGCTAGAGCTCGCTGATCAGCCTCGACTGTGCCTTCTAGTTGCCAGCCATC

1513 TGTGTGTTGCCCTCCCCCGTGCCCTTCCTTGACCTGGAAGGTGCCACTCCAC

poly A  
 1567 TGTCTTTCTTAATAAAATGAGGAAATTGCATCGCATTGTCTGAGTAGGTGTCA

BbsI  
 1621 TTCTATTCTGGGGGTGGGGTGGGGCAGGACAGCAAGGGGAGGATTGGGAAGA

1675 CAATAGCAGGCATGCTGGGGATCGGGTGGGCTCTATGGCTCTGAGCGGAAAG

1729 AACCAGTGGCGTAATACGGTTATCCACAGAATCAGGGGATAACGCAGGAAAGA  
 AflIII

1783 ACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGCGCGTTGC

1837 TGGCGTTTTCCATAGGCTCCGCCCCCTGACGAGCATCAAAAAATCGACGCT

1891 CAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAGATACCAGGCGTTTCCC

1945 CTGGAAGCTCCCTCGTGCGCTCTCTGTTCCGACCTGCCGCTTACCGGATACC

1999 TGTCCGCTTTCTCCCTTCGGGAAGCGTGGCGCTTCTCATAGCTCAGCTGTA

ApaLI  
 2053 GGTATCTCAGTTCGGTGTAGGTCGTCGCTCCAAGCTGGGCTGTGTGCACGAAC

Col E1  
 2107 CCCCCGTCAGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCA

AlwNI  
 2161 ACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTA

2215 GCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTCTTGAAAGTGGTGGCCTAACT

2269 ACGGCTACACTAGAAGGACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTA

2323 CCTTCGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAAACCAACCGCTGGTA

Fig. 2 (cont'd II)

09889182 PAT 68650

09/889182

10/18

2377 GCGGTGGTTTTTTTGTGCAAGCAGCAGATTACGCGCAGAAAAAAGGATCTC  
2431 AAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGGAAACGAAACT  
BspHI  
2485 CACGTTAAGGGATTTTGGTCATGAGATTATCAAAAAGGATCTTCACCTAGATCC  
Bsu36I  
2539 TTTTAAATTAATAATGAAGTTTAAATCAATCTAAAGTATATATGAGTAACCTG  
EcoO109I  
2593 AGGCTATGGCAGGGCCTGCCGCCCCGACGTTGGCTGCGAGCCCTGGGCCCTTAC  
AlwNI  
2647 CCGAACTTGGGGGTGGGGTGGGGAAGGAAGAAACGCGGGCGTATTGGCCCC  
2701 AATGGGGTCTCGGTGGGGTATCGACAGAGTGCCAGCCCTGGGACCGAACCCCGC  
TK poly A  
2755 GTTATGAACAAACGACCAACACCGTGCGTTTTATTCTGTCTTTTATTGCCG  
2809 TCATAGCGCGGGTTCCTTCCGGTATTGTCTCCTTCCGTGTTTCAGTTAGCCTCC  
AvrII  
2863 CCCTAGGGTGGGCGAAGAACTCCAGCATGAGATCCCCGCGCTGGAGGATCATCC  
2917 AGCCGGCGTCCCGGAAACGATTCCGAAGCCCAACCTTTCATAGAAGGCGGCGG  
BstBI  
2971 TGGAAATCGAAATCTCGTGATGGCAGGTTGGGCGTCGTTGGTCGGTCATTTTGA  
3025 ACCCCAGAGTCCCGCTCAGAAGAACTCGTCAAGAAGGCGATAGAAGGCGATGCG  
2634...PhePheGluAspLeuLeuArgTyrPheAlaIleArg  
3079 CTGCGAATCGGGAGCGCGGATACCGTAAAGCAGAGGAGCGGTGAGCCCATTC  
2504GlnSerAspProAlaAlaIleGlyTyrLeuValLeuPheArgAspAlaTrpGlu  
SapI RsrII  
3133 GCCGCCAAGCTCTTCAGCAATATCACGGGTAGCCAACGCTATGTCCTGATAGCG  
2324GlyGlyLeuGluGluAlaIleAspArgThrAlaLeuAlaIleAspGlnTyrArg  
3187 GTCCGCCACACCCAGCCGCCACAGTCGATGAATCCAGAAAAGCGGCCATTTTC  
2144AspAlaValGlyLeuArgGlyCysAspIlePheGlySerPheArgGlyAsnGlu  
3241 CACCATGATATTGGGCAAGCAGGCATCGCCATGGGTACGACGAGATCCTCGCC  
1964ValMetIleAsnProLeuCysAlaAspGlyHisThrValValLeuAspGluGly  
3295 GTCGGCATGTCTCCCTTGAGCCTGGCGAACAGTTTCGGCTGGCGCGAGCCCTG  
1784AspProMetSerAlaLysLeuArgAlaPheLeuGluAlaProAlaLeuGlyGln  
3349 ATGCTCTTGATCATCTGATCGACAAGACCGGCTTCCATCCGAGTACGTGCTCG  
1604HisGluGlnAspAspGlnAspValLeuGlyAlaGluMetArgThrArgAlaArg  
3403 CTCGATGCGATGTTTCGCTTGGTGGTGAATGGGCGAGTAGCCGGATCAAGCGT  
1424GluIleArgHisLysAlaGlnHisAspPheProCysThrAlaProAspLeuThr  
3457 ATGCAGCCGCCGATTGTCATCAGCCATGATGGATACTTCTCGGCAGGAGCAAG  
1244HisLeuArgArgMetAlaAspAlaMetIleSerValLysGluAlaProAlaLeu  
3511 GTGAGATGACAGGAGATCCTGCCCGGCACTTCGCCCAATAGCAGCCAGTCCCT  
1064HisSerSerLeuLeuAspGlnGlyProValGluGlyLeuLeuLeuTrpAspArg  
FspI Neo-R.  
Tth111I PvuII MscI  
3565 TCCCGCTTCAGTGACAACGTGAGCAGCTGCGCAAGGAACGCCCGTCGTGGC  
884GlyAlaGluThrValValAspLeuValAlaAlaCysProValGlyThrThrAla  
3619 CAGCCACGATAGCCGCGCTGCCTCGTCTTGCAAGTTATTGAGGACCCGACAG  
704LeuTrpSerLeuArgAlaAlaGluAspGlnLeuGluAsnLeuAlaGlySerLeu  
NarI  
3673 GTCGGTCTTGACAAAAAGAACCGGGCGCCCTGCGCTGACAGCCGGAACACGGC  
524AspThrLysValPheLeuValProArgGlyGlnAlaSerLeuArgPheValAla  
3727 GGCATCAGACAGCCGATTGTCTGTTGTGCCCAGTCATAGCCGAATAGCCTCTC  
344AlaAspSerCysGlyIleThrGlnGlnAlaTrpAspTyrGlyPheLeuArgGlu  
3781 CACCAAGCGCGCGGAGAACCTGCGTGCAATCCATCTTGTTCATCATGCGAAA  
164ValTrpAlaAlaProSerGlyAlaHisLeuGlyAspGlnGluIleMet  
StuI  
BsaBI AvrII  
3835 CGATCTCATCTGTCTCTTGATCGATCTTTGAAAAGCCTAGGCCTCCAAAAA

Fig. 2 (cont'd III)

09/889182

11/1B

3889 BseRI AGCCTCCTCACTACTTCTGGAATAGCTCAGAGGCCGAGGAGGCGGCTCGGCCT BseRI  
 3943 CTGCATAAATAAAAAAATTAGTCAGCCATGGGGCGGAGAATGGGCGGAAGTGG  
 3997 SV40 ori & Promotor GCGGAGTTAGGGGCGGGATGGGCGGAGTTAGGGGCGGACTATGGTTGCTGACT  
 4051 NsiI AATTGAGATGCATGCTTTGCATACTTCTGCCTGCTGGGGAGCCTGGGGACTTTC  
 4105 SexAI CACACCTGGTTGCTGACTAATTGAGATGCATGCTTTGCATACTTCTGCCTGCTG NsiI  
 4159 PvuII GGGAGCCTGGGGACTTTCCACACCCTAACTGACACACATTCCACAGCTGGTTC  
 4213 Bsu36I TTCGGCCTCAGGACTCTTCCTTTTCAATAAATCAATCTAAAGTATATATGAGT  
 4267 AAACCTTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGCACCTATCTCAGCGA  
 2874...TrpHisLysIleLeuSerAlaGlyIleGluAlaIle  
 4321 Eam1105I TCTGTCTATTTCTGTTCCATAGTTGCCTGACTCCCCGTCGTGTAGATAACTA  
 2744eGlnArgAsnArgGluAspMetThrAlaGlnSerGlyThrThrTyrIleValVal  
 4375 CGATACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCAATGATACCGCGAGACC  
 2564IleArgSerProLysGlyAspProGlyLeuAlaAlaIleIleGlyArgSerGly  
 4429 CACGCTCACC GGCTCCAGATTTATCAGCAATAAACAGCCAGCCGGAAGGGCCG  
 2384yArgGluGlyAlaGlySerLysAspAlaIlePheTrpGlyAlaProLeuAlaSe  
 4483 AGCGCAGAGTGGTCTGCAACTTTATCCGCTCCATCCAGTCTATTAATTGTT  
 2204rArgLeuLeuProGlyAlaValLysAspAlaGluMetTrpAspIleLeuGlnGly  
 4537 FspI Psp1406I GCCGGGAAGCTAGAGTAAGTAGTTCGCCAGTTAATAGTTTGCACAAGCTTGTG  
 2024nArgSerAlaLeuThrLeuLeuGluGlyThrLeuLeuLysArgLeuThrThrAla  
 4591 CCATTGCTACAGGCATCGTGGTGTACGCTCGTCTGTTGGTATGGCTTCATTCA  
 1844aMetAlaValProMetThrThrAspArgGluAspAsnProIleAlaGluAsnLeu  
 4645 GTCCTGGTTCCCAACGATCAAGCGGAGTTACATGATCCCCATGTTGTGCAAAA  
 1664uGluProGluTrpArgAspLeuArgThrValHisAspGlyMetAsnHisLeuPh  
 4699 PvuI AAGCGGTAGCTCCTTCGGTCTCCGATCGTTGTGCAAGTAAGTTGGCCGAG  
 1484eAlaThrLeuGluLysProGlyGlyIleThrThrLeuLeuLeuAsnAlaAlaTh  
 4753 TGTATCACTCATGTTATGGCAGCACTGCATAATTCTCTTACTGTCTATGCCAT  
 1304rAsnAspSerMetThrIleAlaAlaSerCysLeuGluArgValThrMetGlyAs  
 4807 bla CCGTAAGATGCTTTTCTGTGACTGGTGAGTACTCAACCAAGTCATTCTGAGAAT ScaI  
 1124pThrLeuHisLysGluThrValProSerTyrGluValLeuAspAsnGlnSerTy  
 4861 AGTGTATGCGGCGACCGAGTTGCTCTTGCCGCGCTCAATACGGGATAATACCG  
 944rHisIleArgArgGlyLeuGlnGluGlnGlyAlaAspIleArgSerLeuValAla  
 4915 Psp1406I CGCCACATAGCAGAACTTTAAAGTGCTCATCATTGGAACAGTTCTTCGGGGC  
 764aGlyCysLeuLeuValLysPheThrSerMetMetProPheArgGluGluProAr  
 4969 GAAAACTCTCAAGGATCTTACCCTGTTGAGATCCAGTTCGATGTAACCCACTC  
 584gPheSerGluLeuIleLysGlySerAsnLeuAspLeuGluIleTyrGlyValAr  
 5023 ApaI GTGCACCACTGATCTTCAGCATCTTTACTTTACCAGCGTTTCTGGGTGAG  
 404gAlaGlyLeuGluAspGluAlaAspLysValLysValLeuThrGluProHisAla  
 5077 CAAAAACAGGAAGGCAAAATGCCGCAAAAAGGGAATAAGGGCGACACGGAAT  
 224aPheValProLeuCysPheAlaAlaPhePheProIleLeuAlaValArgPheHis  
 5131 SspI GTTGAATACTCACTCTTCTCTTTTCAATATTATTGAAGCATTTATCAGGGTT  
 44sGlnIleSerMet  
 5185 BspHI ATTGTCTCATGAGCGGATACATATTTGAATGTATTTAGAAAAATAACAAATAG

Fig. 2 (cont'd IV)

09/889182

12/18

5239 GGGTTCGCGCACATTTCCCGAAAAGTGCCACCTGACGCGCCCTGTAGCGGCG

5293 CATTAAAGCGCGGGGGTGTGGTGGTTACGCGCAGCGTGACCGCTACACTTGCCA

5347 GCGCCCTAGCGCCCGCTCCTTTCGCTTCTTCCCTTCCTTCTCGCCACGTTCCG

5401 CCGGCTTTCCCGTCAAGCTCTAAATCGGGGGCTCCCTTAGGGTTCGATTTA

5455 GTGCTTTACGGCACCTCGACCCCAAAAACTTGATTAGGGTGATGGTTCACGTA

5509 GTGGGCCATCGCCCTGATAGACGGTTTTTCGCCCTTGACGTTGGAGTCCACGT

5563 TCTTTAATAGTGGACTCTTGTTCAAACTGGAACAACACTCAACCTATCTCGG

5617 TCTATTCTTTGATTATATAAGGGATTTTGGCGATTTCGGCCTATTGGTTAAAAA

5671 ATGAGCTGATTTAACAAAAATTAACGCGAATTTTAACAAATATTAACGCTTA

5725 CAATTAC

Stem loop A

f1 IR Stem loop B

DraIII

Stem loop C Primer-RNA Start Transcription VS-Synthesis

Nicking site Stem loop D Stem loop E

SspI

Fig. 2 (cont'd V)

09889182-011002



09/889182

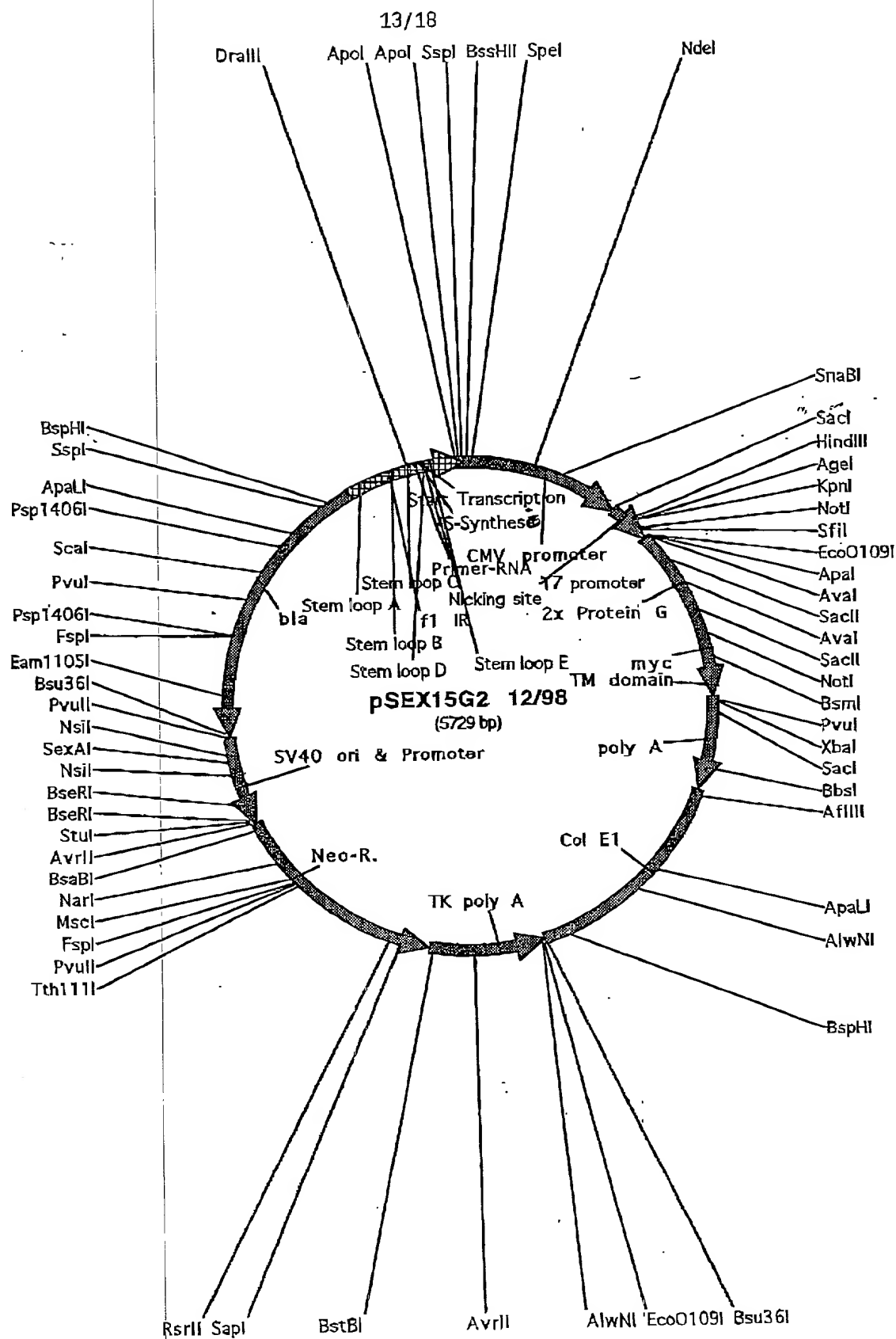


Fig. 3

09/889182

14/18

BssHII                      SphI  
 1 GCGCGCGTTGACATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTCA  
 57 TTAGTTCATAGCCCATATATGGAGTTCGCGTTACATAACTTACGGTAAATGGCCC  
 113 GCCTGGCTGACCGCCCAACGACCCCGCCATTGACGTCAATAATGACGTATGTTT  
 169 CCATAGTAACGCCAATAGGGACTTTCATTGACGTCAATGGGTGGACTATTTACGG  
 NdeI  
 225 TAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTAT  
 CMV promoter  
 281 TGACGTCAATGACGGTAAATGGCCCGCTGGCATTATGCCAGTACATGACCTTAT  
 SnaBI  
 337 GGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGTG  
 393 ATGCGGTTTTGGCAGTACATCAATGGGCGTGGATAGCGGTTTGACTCACGGGGATT  
 449 TCCAAGTCTCCACCCATTGACGTCAATGGGAGTTGTTTGGCAGCAAAATCAAC  
 505 GGGACTTTCCAAAATGTCGTAACTCCGCCCCATTGACGCAATGGGCGGTAGG  
 SacI  
 561 CGTGTACGGTGGGAGGTCTATATAAGCAGAGCTCTCTGGCTAACTAGAGAACCAC  
 T7 promoter                      HindIII KpnI  
 617 TGCTTACTGGCTTATCGAAATTAATACGACTCACTATAGGGAGACCAAGCTTGGT  
 SfiI  
 AgeI                      NotI  
 673 ACCGGTGGCGATGGCACCTGCTGCTGCTCCTGCTGTTGGCGCGCCCTGGCCCC  
 1 MetAlaProCysMetLeuLeuLeuLeuLeuAlaAlaAlaLeuAlaPr  
 ApaI  
 EcoO109I                      Aval  
 729 GACTCAGACCCGCGGGGGCCAAAAGCCGAGGTGATCGATGCCAGCGAGCTGA  
 16 Thr Glu Thr Arg Ala Glu Ala Glu Lys Pro Glu Val Ile Asp Ala Ser Glu Leu T  
 785 CCCCCGCGTGACCACTACAAGCTAGTGATCAACGGCAAGACCCTGAAGGGCGAG  
 35 Thr Pro Ala Val Thr Thr Tyr Lys Leu Val Ile Asn Gly Lys Thr Leu Lys Gly Glu  
 SacII  
 841 ACCACCACCGAGGCGGTGGACGCGCCACCGCGGAGAAGGTGTTCAAACAATACGC  
 54 Thr Thr Thr Glu Ala Val Asp Ala Ala Thr Ala Glu Lys Val Phe Lys Glu Tyr Ala  
 897 TAATGACAACGGGGTGGACGGCGAGTGGACTTACGACGACGCCACCAAGACCTTCA  
 72 Asn Asp Asn Gly Val Asp Gly Glu Trp Thr Tyr Asp Asp Ala Thr Lys Thr Phe T  
 Aval  
 2x Protein G  
 953 CCGTGACCGAGAAGCCCGAGGTGATCGATGCCAGCGAGCTGACCCCGCGGTGACC  
 91 Thr Val Thr Glu Lys Pro Glu Val Ile Asp Ala Ser Glu Leu Thr Pro Ala Val Thr  
 1009 ACCTACAAGCTAGTGATCAACGGCAAGACCCTGAAGGGCGAGACCAACCGAGGC  
 110 Thr Tyr Lys Leu Val Ile Asn Gly Lys Thr Leu Lys Gly Glu Thr Thr Thr Glu Ala  
 SacII  
 1065 CGTGGACGCGCCACCGCGGAGAAGGTGTTCAAACAATACGCTAATGACAACGGGG  
 128 Val Asp Ala Ala Thr Ala Glu Lys Val Phe Lys Glu Tyr Ala Asn Asp Asn Gly V

Fig. 3 (cont'd I)

09/889182

15/18

NotI  
 1121 TCGACGGCGAGTGGACTTACGACGACGCCACCAAGACCTTCACCGTGACCGAGGCG  
 1477 alAspGlyGluTrpThr TyrAspAspAlaThrLysThrPheThrValThrGluAla

myc  
 1177 GCCGCAGAACAAAACTCATCTCAGAAGAGGATCTGAATGGGGCCGTCGACGAACA  
 1667 AlalalGluGlnLysLeu||eSerGluGluAspLeuAsnGlyAlaValAspGluGlu

BsmI  
 1233 AAAACTCATCTCAGAAGAGGATCTGAATGCTGTGGGCCAGGACACGCAGGAGGTCA  
 1847 nLysLeu||eSerGluGluAspLeuAsnAlaValGlyGlnAspThrGlnGluVal||

1289 TCGTGGTGCCCACTCCTTGCCCTTTAAGGTGGTGGTGATCTCAGCCATCCTGGCC  
 2037 leValValProHisSerLeuProPheLysValValVal||eSerAlalleLeuAla

TM domain  
 1345 CTGGTGGTGCTCACCATCATCTCCCTTATCATCCTCATCATGCTTTGGCAGAAGAA  
 2227 LeuValValLeuThr||elleSerLeu||elleLeu||eMetLeuTrpGlnLysLys

PvuI XbaI  
 1401 GCCACGTTTCGTCGGCCGATCGAGAATCCATCTAGAGCTATTCTATAGTGTCACCTA  
 2407 sProArgSerSerAlaAspArgGluSer||e\*\*\* ←

SacI  
 1457 AATGCTAGAGCTCGCTGATCAGCCTCGACTGTGCCTTCTAGTTGCCAGCCATCTGT  
 ←

poly A  
 1513 TGTTTGCCCTCCCCCGTGCCTTCCTTGACCCTGGAAGGTGCCACTCCCACTGTCC  
 1569 TTTCTAATAAAATGAGGAAATTGCATCGCATTGTCTGAGTAGGTGTCATTCTATT

BbsI  
 1625 CTGGGGGGTGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGACAATAGCAG  
 1681 GCATGCTGGGGATGCGGTGGGCTCTATGGCTTCTGAGGCGGAAAGAACCACTGGCG

AflIII  
 1737 GTAATACGGTTATCCACAGAATCAGGGGATAACCGAGGAAAGAACATGTGAGCAAA  
 1793 AGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCTTGTGGCGTTTTTCATA

1849 GGCTCCGCCCCCTGACGAGCATCAGAAAATCGACGCTCAAGTCAGAGGTGGCGA  
 1905 AACCCGACAGGACTATAAGATACAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCG  
 1961 CTCTCTGTTCGACCCCTGCCGCTTACCGGATACCTGTCCGCTTCTCCCTCGG  
 2017 GAAGCGTGGCGCTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCCGTGTAGGT

ApaI Col E1  
 2073 GTTCGCTCCAAGCTGGGCTGTGTGCAAGAACCCCGTTACGCCGACCGCTGCGC  
 2129 CTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCAC

AlwNI  
 2185 TGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACA  
 2241 GAGTTCCTGAAGTGGTGGCTAACTACGGCTACACTAGAAGGACAGTATTTGGTAT  
 2297 CTGGGCTCTGCTGAAGCCAGTTACCTTCGAAAAAGAGTTGGTAGCTCTTGATCCG  
 2353 GCAACAAACCAACCGCTGGTAGCGGTGTTTTTTTGTGCAAGCAGCAGATTACG  
 2409 CGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTCTACGGGGTCTGACGC

Fig. 3 (cont'd II)

09889182-01002

09/889182

16/18

2465 TCAGTGGAAACGAAAACCTCACGTTAAGGGATTTTGGTCATGAGATTATCAAAAAGGA  
2521 TCTTCACCTAGATCCTTTTAAATTAAAAATGAAGTTTAAATCAATCTAAAGTATA

BspHI  
EcoQ109I  
Bsu36I AlwNI  
2577 TATGAGTAACCTGAGGCTATGGCAGGGCCTGCCGCCCGACGTTGGCTGCCAGCCC

2633 TGGGCCCTTCACCCGAATTGGGGGGTGGGGTGGGGAAAAAGGAAGAAACGCGGGCGT

2689 ATTGGCCCCAATGGGGTCTCGGTGGGGTATCGACAGAGTGCCAGCCCTGGGACCGA

TK poly A  
2745 ACCCCGCGTTTATGAACAAACGACCAACACCGTGCGTTTATTCTGTCTTTTAT

2801 TGCCGTCATAGCGCGGGTTCCTTCCGGTATTGTCTCCTTCCGTGTTTCAGTTAGCC

AvrII  
2857 TCCCCCTAGGGTGGGCGAAGAACTCCAGCATGAGATCCCCGCGCTGGAGGATCATC

2913 CAGCCGGCGTCCCGGAAAACGATTCCGAAGCCCAACCTTTCATAGAAGGCGGCGGT

BstBI  
2969 GGAATCGAAATCTCGTGATGGCAGGTTGGGCGTCGCTTGGTCGGTCAITTCGAACC  
3025 CCAGAGTCCCGCTCAGAAGAACTCGTCAAGAAAGCGATAGAAGGCGATGCGCTGCG

2634 \*\*\*PhePheGluAspLeuLeuArgTyrPheAlaIleArgGlnSer  
3081 AATCGGGAGCGGCGATACCGTAAAGCAGGAGGAAGCGGTGAGCCCATTCGCCGCCA  
2481 rAspProAlaAlaIleGlyTyrLeuValLeuPheArgAspAlaTrpGluGlyGlyL

SapI RsrII  
3137 AGCTCTTCAGCAATATCACGGGTAGCCAACGCTATGTCCTGATAGCGGTCCGCCAC  
2291 euGluGluAlaIleAspArgThrAlaLeuAlaIleAspGlnTyrArgAspAlaVal  
3193 ACCCAGCCGGCCACAGTCGATGAATCCAGAAAAGCGGCCATTTCCACCATGATAT  
2111 GlyLeuArgGlyCysAspIlePheGlySerPheArgGlyAsnGluValMetIleAs  
3249 TCGGCAAGCAGGCATCGCCATGGGTCAAGCAGATCCTCGCGTCGGGCATGCTC  
1921 nProLeuCysAlaAspGlyHisThrValValLeuAspGluGlyAspProMetSerA  
3305 GCCTTGAGCCTGGCGAACAGTTCCGGCTGCGCGCAGCCCTGATGCTCTGATCATC  
1731 laLysLeuArgAlaPheLeuGluAlaProAlaLeuGlyGlnHisGluGlnAspAsp  
3361 CTGATCGACAAAGCCGCTTCCATCCGAGTACGTGCTCGCTCGATGGGATGTTTCG  
1551 GluAspValLeuGlyAlaGluMetArgThrArgAlaArgGluIleArgHisLysAl  
3417 CTGGTGGTCAATGGGCAGGTAGCCGGATCAAGCGTATGACGCCGCCGATTGCA  
1361 aGlnHisAspPheProCysThrAlaProAspLeuThrHisLeuArgArgMetAlaA  
3473 TCAGCCATGATGGATACTTCTCGGCAGGAGCAAGGTGAGATGACAGGAGATCCTG  
1171 spAlaMetIleSerValLysGluAlaProAlaLeuHisSerSerLeuLeuAspGln

Tth111I  
3529 CCCCCGCACTTCGCCCAATAGCAGCCAGTCCCTTCCCGCTTCAGTGACAACGTCGA  
991 GlyProValGluGlyLeuLeuLeuTrpAspArgGlyAlaGluThrValValAspLe

Neo-R.  
PvuIIFspI MscI  
3585 GCACAGCTGCGCAAGGAACGCCCGTCGTGGCCAGCCACGATAGCCGGGCTGCCTCG  
801 uValAlaAlaCysProValGlyThrThrAlaLeuTrpSerLeuArgAlaAlaGluA

NarI  
3641 TCTTGCAAGTTCAATGAGGACCGGACAGGTGGTCTTGACAAAAAGAACCGGGCG  
611 spGlnLeuGluAsnLeuAlaGlySerLeuAspThrLysValPheLeuValProArg  
3697 CCCCTGCGTGACAGCCGGAACACGGCGGCATCAGAGCAGCCGATTGTCTGTTGTG  
431 GlyGlnAlaSerLeuArgPheValAlaAlaAspSerCysGlyIleThrGlnGlnAl  
3753 CCCAGTCATAGCCGAATAGCCTCTCCACCCAAGCGGCCGAGAACCTGCGTGCAAT  
241 aTrpAspTyrGlyPheLeuArgGluValTrpAlaAlaProSerGlyAlaHisLeuG

BsaBI  
3809 CCATCTTGTTCAATCATGCGAAACGATCCTCATCTGTCTCTTGATCGATCTTTGC  
511 yAspGlnGluIleMet

StuI  
AvrII BseRI  
3865 AAAAGCCTAGGCCTCCAAAAAGCCTCCTCACTACTTCTGGAATAGCTCAGAGGCC

Fig. 3 (cont'd III)

09/889182

17/18

BseRI  
3921 GAGGAGGGCGGCTCGGCCTCTGCATAAATAAAAAAATTAGTCAGCCATGGGGCGG

SV40 ori & Promoter  
3977 AGAATGGGCGGAAGTGGGCGGAGTTAGGGGCGGATGGGCGGAGTTAGGGGCGGA

NsiI  
4033 CTATGGTTGCTGACTAATTGAGATGCATGCTTTGCATCTTCTGCTGCTGGGGAG

SexAI NsiI  
4089 CCTGGGGACTTTCCACACCTGGTTGCTGACTAATTGAGATGCATGCTTTGCATACT

PvuII  
4145 TCTGCTGCTGGGGAGCCTGGGGACTTTCCACACCCTAACTGACACACATTCCACA

Bsu36I  
4201 GCTGGTCTTTCCGCCCTCAGGACTCTTCCTTTTCAATAAATCAATCTAAAGTATA  
4257 TATGAGTAAACTTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGCACCTATCTC  
2874...TrpHisLysIleLeuSerAlaGlyIleGlu<sup>+</sup>  
Eam110SI  
4313 AGCGATCTGTCTATTTTCGTTTCATCCATAGTTGCCTGACTCCCGTCTGTAGATAA  
2764AlaIleGlnArgAsnArgGluAspMetThrAlaGlnSerGlyThrThrTyrIleVal  
4369 CTACGATACGGGAGGCTTACCATCTGGCCCCAGTGCTGCAATGATACCGCGAGAC  
2574IValIleArgSerProLysGlyAspProGlyLeuAlaAlaIleIleGlyArgSerG  
4425 CCACGCTCACCAGCTCCAGATTTATCAGCAATAAACAGCCAGCCGGGAAGGGCCGA  
2384IyArgGluGlyAlaGlySerLysAspAlaIlePheTrpGlyAlaProLeuAlaSer  
4481 GCGCAGAAGTGGTCTGCAACTTTATCCGCTCCATCCAGTCTATTAAATTGTGCC  
2204ArgLeuLeuProGlyAlaValLysAspAlaGluMetTrpAspIleLeuGlnGlnAr  
FspI Psp1406I  
4537 GGGAAAGCTAGAGTAAGTAGTTCCGCGTAAATAGTTTGGCAACGTTGTTGCCATT  
2014gSerAlaLeuThrLeuLeuGluGlyThrLeuLeuLysArgLeuThrThrAlaMetA  
4593 GCTACAGGCATCGTGGTGTACGCTCGTCTTGGTATGGCTTCATTCAGCTCCGG  
1824IaValProMetThrThrAspArgGluAspAsnProIleAlaGluAsnLeuGluPro  
4649 TTCCCAACGATCAAGGCGAGTTACATGATCCCCATGTTGTGCAAAAAGCGGTTA  
1644GluTrpArgAspLeuArgThrValHisAspGlyMetAsnHisLeuPheAlaThrLe  
PvuI  
4705 GCTCCTTCGGTCTCCGATCGTTGTCAGAAAGTAAGTTGGCCGAGTGTATCACTC  
1454uGluLysProGlyGlyIleThrThrLeuLeuLeuAsnAlaAlaThrAsnAspSerM  
bla  
4761 ATGGTTATGGCAGCACTGCATAATTCTCTTACTGTCATGCCATCCGTAAGATGCTT  
1264etThrIleAlaAlaSerCysLeuGluArgValThrMetGlyAspThrLeuHisLys  
ScaI  
4817 TTCTGTGACTGGTGAGTACTCAACCAAGTCATTCTGAGAATAGTGTATGCGGCGAC  
1084GluThrValProSerTyrGluValLeuAspAsnGlnSerTyrHisIleArgArgGlu  
4873 CGAGTTGCTCTTCCCGCGGTCAATACGGGATAATACCGCGCCACATAGCAGAACT  
894yLeuGlnGluGlnGlyAlaAspIleArgSerLeuValAlaGlyCysLeuLeuValL  
Psp1406I  
4929 TTAAGAGTGCTCATCTTGGAAAACGTTCTTCGGGGCGAAAACCTCAAGGATCTT  
704ysPheThrSerMetMetProPheArgGluGluProArgPheSerGluLeuIleLys  
ApaI  
4985 ACCGCTGTTGAGATCCAGTTCGATGTAACCCACTCGTGCACCCAACTGATCTTCAG  
524GlySerAsnLeuAspLeuGluIleTyrGlyValArgAlaGlyLeuGlnAspGluAl  
5041 CATCTTTTACTTTCACAGCGTTTCTGGGTGAGCAAAAACAGGAAGGCAAAATGCC  
334aAspLysValLysValLeuThrGluProHisAlaPheValProLeuCysPheAlaA  
5097 GCAAAAAGGGAATAAGGGCGACACGGAAATGTTCAATACTCATCTCTTCTTTT  
144IaPhePheProIleLeuAlaValArgPheHisGlnIleSerMet  
SspI BspHI  
5153 TCAATATTATTGAAGCATTTATCAGGGTTATTGCTCATGAGCGGATACATATTTG  
5209 AATGTATTTAGAAAAATAAACAAATAGGGGTTCCGCGCACATTTCCCCGAAAAGTG  
5265 CCACCTGACGCGCCTGTAGCGGCGATTAAAGCGCGCGGGTGTGGTGGTTACGCG

Fig. 3 (cont'd IV)

09/889182

18/18

Stem loop A  
 5321 CAGCGTGACCGCTACACTTGCCAGCGCCCTAGCGCCCGCTCCTTTCGCTTTCTTCC  
 -----  
 5377 CTTCCTTCTCGCCACGTTCCCGGCTTCCCCGTCAAGCTCTAAATCGGGGGCTC  
 -----  
 f1 IR Stem loop B  
 5433 CCTTTAGGGTCCGATTAGTGCTTACGGCACCTCGACCCCAAAAACTTGATTA  
 -----  
 DraIII Stem loop C Primer-RNA  
 5489 GGGTGATGGTTCACGTAGTGGGCCATCGCCCTGATAGACGGTTTTTCGCCCTTGA  
 -----  
 Start Transcription  
 VS-Synthesis Nicking site Stem loop D Stem loop E  
 5545 CGTTGGAGTCCACGTTCTTTAATAGTGGA CTCTGTCCAACTGGAACAACACTC  
 -----  
 5601 AACCCATCTCGGTCTATTCTTTTGATTTATAAGGGATTTGCCGATTTGGGCCTA  
 -----  
 Apol Apol SspI  
 5657 TTGGTAAAAAATGAGCTGATTTAACAAAAATTTAACGCGAATTTTAACAAATAT  
 -----  
 5713 TAACGCTTACAATTTAC  
 -----

Fig. 3 (cont'd V)

0989182-01002